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APPLICATION FOR A
PRODUCT AUTHORISATION
FOR

HIGH POTENCY FACTORATE

AP000582

Armour Pharmaceutical Company Limited, Eastbourne, Sussex

ARMOUR000787

APPLICATION FOR A PRODUCT AUTHORISATION FOR

H I G H P O T E N C Y F A C T O R A T E

CONTENTS

WJT/JJ

ARMOUR PHARMACEUTICAL COMPANY LIMITED,
HAMPDEN PARK,
EASTBOURNE,
EAST SUSSEX,
BN22 9AG

NOVEMBER 1981

AP000583

APPLICATION FOR A PRODUCT AUTHORISATION FOR

H I G H P O T E N C Y F A C T O R A T E

PART I SUMMARY

1. NAME AND ADDRESS OF APPLICANT

Armour Pharmaceutical Company,
Hampden Park,
Eastbourne,
U.K.

2. NAME AND ADDRESS OF PROPOSED HOLDER OF AUTHORISATION

As in 1. above.

3. NAME AND ADDRESS OF PERSON RESIDENT IN IRELAND

Berk Pharmaceuticals Limited,
Dublin Industrial Estate,
Glasnevin,
Dublin 2.

4. ROLE OF PROPOSED HOLDER OF AUTHORISATION

(a) As person responsible for composition of the product in Ireland.

(b) As person who imports or procures its importation.

5. NAME AND ADDRESS OF ACTUAL IMPORTER

Berk Pharmaceuticals Limited,
Dublin Industrial Estate,
Glasnevin,
Dublin 2.

6. ACTIVITIES FOR WHICH THE AUTHORISATION IS REQUIRED

(a) Selling or supplying the product in Ireland.

(b) Importing or procuring the importation of the product.

7. PROPRIETARY NAME OF THE PRODUCT

High Potency Factorate

8. PRODUCT FORM

Vials of lyophilised powder for intravenous injection after reconstitution with Water for Injections B.P.

9. ACTIVE CONSTITUENT

Antihaemophilic Factor (Human).

10. CLINICAL USE

Treatment of Haemophilia A resulting from deficiency of Antihaemophilic Factor (Factor VIII).

11. RECOMMENDED DOSAGE

Dosage should be adjusted according to the patient's individual needs. Generally one unit of Factor VIII activity per kg will increase circulating Factor VIII level by 2%.

The following general doses are suggested:

(1) Overt bleeding Initially 20 units per kg of bodyweight followed by 10 units per kg every eight hours for the first 24 hours and the same dose every 12 hours for the next 3 or 4 days. For massive wounds give until bleeding stops and maintain with 20 units per kg 8-hourly to achieve a minimum Factor VIII level of 40%.

(2) Muscle Haemorrhages

(a) Minor haemorrhages in extremities or non-vital areas: 10 units per kg one a day for 2 - 3 days.

(b) Massive haemorrhage in non-vital areas: 10 units per kg by infusion at 12 hour intervals for 2 days and then once daily for a further 2 days.

(c) Haemorrhage near vital organs (Neck, throat, subperitoneal): 20 units per kg initially; then 10 units per kg every 8 hours. After 2 days the dose may be reduced by one half.

(3) Joint Haemorrhages

10 units per kg every 8 hours for a day; then twice daily for 1 or 2 days. If aspiration is carried out, 10 units per kg just prior to aspiration with additional infusions of 10 units per kg 8 hours later and again on the following day.

(4) Surgery

Dosages of 30 - 40 units per kg bodyweight prior to surgery are recommended. After surgery 20 units per kg every 8 hours should be administered. Close laboratory control to maintain the blood level of Factor VIII above 40% of normal for at least 10 days post operatively is suggested.

(5) Dental Extractions

For simple extractions a pre-operative dose of 20 - 25 units per kg, sufficient to raise the Factor VIII level to 50% should be given, followed by intravenous administration of tranexamic acid. For multiple extractions further doses of Factor VIII may be advisable 24 or 36 hours after the operation.

12. SIDE EFFECTS, CONTRA-INDICATIONS, PRECAUTIONS AND WARNINGS

Warnings

Factor VIII is prepared from human plasma, each donation of which has been found negative for hepatitis B surface antigen (HBsAg) by the radioimmunoassay (RIA) method. In addition each batch, after reconstitution as recommended in this leaflet, has been tested and found negative by the RIA method. However since no completely reliable laboratory test is yet available to detect all potentially infectious plasma donations, the risk of transmitting viral hepatitis is still present.

Side-Effects

Products of this type are known to cause mild chills, nausea or stinging at the infusion point.

Precautions

Factor VIII contains low levels of group A and B isohaemagglutinins. When large volumes are given to patients of blood groups A, B or AB, the possibility of intravascular haemolysis should be considered. Such patients should be monitored by means of haematocrit and direct Coombs test for signs of progressive anaemia.

Contra-Indications

There are no known contra-indications to antihaemophilic fraction.

13. METHOD OF RETAIL SALE OR SUPPLY

To hospitals only.

14. METHOD OF SALES PROMOTION

Via the professions as a prescription item.

15. MANUFACTURE OF DOSAGE FORM

Manufacture of the dosage form will be carried out by Armour Pharmaceutical Company, P.O. Box 511, Kankakee, Illinois 60901, U.S.A. Assembly of the product into final containers will be carried out at Armour Pharmaceutical Company Limited, Hampden Park, Eastbourne. Vials of Water for Injections BP supplied in Home Treatment Packs for reconstitution of the product will be supplied by Phoenix Pharmaceuticals, Phoenix Estate, Caerphilly Road, Cardiff, Wales, CF4 4XG.

16. QUALITY CONTROL

Quality control in-process and on the finished product will be exercised by Armour Pharmaceutical Company, Kankakee, USA.

The responsibility for release of the product, based on batch analysis data supplied with each batch, will rest on the Quality Control Manager at Armour Pharmaceutical Company Limited, Eastbourne.

17. CONTAINERS

High Potency Factorate is supplied in 50 ml (500 or 1000 iu/vial) or 30 ml (250 iu/vial) Type I glass vials with 20 mm neck finish. The closure is a grey butyl rubber stopper with an aluminium seal and brown non traumatic flip-top cap. Home treatment packs are made available in certain instances and these contain a vial of Water for Injections BP for reconstitution of the product.

18. LABELLING

Texts for product label and package insert are attached.

19. SAMPLE OF PACKAGED PRODUCT

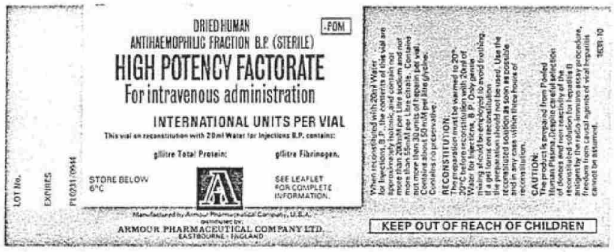
A sample of the finished product is supplied with this documentation.

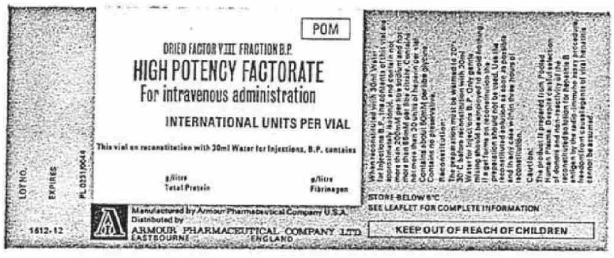
20. MANUFACTURING AUTHORISATION

High Potency Factorate is manufactured by Armour Pharmaceutical Company Kankakee, in accordance with Establishment Licence 149, issued by the United States Department of Health, Education and Welfare. A copy of this document is attached.

21. OTHER MARKETING AUTHORISATIONS

High Potency Factorate is licensed for sale in the United Kingdom under Product Licence No. 0231/0044 granted in June 1979.





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control to maintain the blood level of Factor VIII above 40% of normal for at least 10 days post-operatively is suggested.

5. **Dental Extractions**
For simple extractions a pre-operative dose of 20-25 units per kg sufficient to raise the Factor VIII level to 50% should be given, followed by intravenous administration of tranexamic acid. For multiple extractions further doses of Factor VIII may be advisable 24 or 36 hours after the operation. (Dormandy 1977.)

WARNING

Factor VIII is prepared from human plasma, each donation of which has been found negative for hepatitis B surface antigen (HBsAg) by the radioimmunoassay (RIA) method. In addition, each batch, after reconstitution as recommended in this leaflet, has been tested and found negative by the RIA method. However, since no completely reliable laboratory test is yet available to detect all potentially infectious plasma donations, the risk of transmitting viral hepatitis is still present.

SIDE EFFECTS

Products of this type are known to cause mild chills, nausea or stinging at the infusion site.

PRECAUTIONS

Factor VIII contains low levels of group A and B isohaemagglutinins. When large volumes are given to patients of blood groups A, B or AB, the possibility of intravascular haemolysis should be considered. Such patients should be monitored by means of haematocrit and direct Coombs test for signs of progressive anaemia.

CONTRA-INDICATIONS

There are no known contraindications to antihaemophilic fraction.

STORAGE

HIGH POTENCY FACTORATE is to be stored below 6°C. When stored as directed, it will maintain its labelled potency for the period indicated on the label.

HOW SUPPLIED

HIGH POTENCY FACTORATE is supplied in single dose vials, there being two sizes of vial, dependent on the potency range being used.

DRIED HUMAN ANTIHAEMOPHILIC FRACTION B.P. (STERILE) HIGH POTENCY FACTORATE FOR INTRAVENOUS USE

The potency of each batch of this product is given on the container and package labels. See instructions given under "Recommended Reconstitution" for potency related reconstitution instructions.

Dried Human Antihaemophilic Fraction HIGH POTENCY FACTORATE is a stable lyophilised concentrate of Factor VIII (AHF, AHG) prepared from pooled human plasma intended for use in therapy of classical haemophilia (Haemophilia A).

A hereditary disorder of blood coagulation occurring almost exclusively in males, Haemophilia A results in profuse bleeding in joints, muscles or internal organs as a result of minor trauma. The disease appears to be due to a deficiency of a specific plasma protein, antihaemophilic factor. Factor VIII provides temporary replacement of the missing clotting factor.

Affected individuals frequently require therapy following minor trauma. Surgery, when required in such individuals must be preceded by temporary correction of the clotting abnormality with fresh plasma transfusions, cryoprecipitate or by injections of Factor VIII concentrates. Advantages of the use of concentrates of Factor VIII are the avoidance of hyperproteinaemia, overloading the circulatory system and possible kidney dysfunction resulting from large volume transfusions.

Several different concentrations of Factor VIII have been used successfully. These range from Fraction I of Cohn to highly purified, potent preparations. HIGH POTENCY FACTORATE is a purified preparation with lower levels of fibrinogen and other non-AHF proteins per international unit than "Intermediate Purity" AHF preparations. Upon reconstitution, as directed, HIGH POTENCY FACTORATE contains 15 to 40 times as much Factor VIII as does an equal volume of plasma. Thus it may be used to correct deficiencies in Factor VIII levels without overloading the circulatory system.

COMPOSITION AND STANDARDISATION

Each vial contains the labelled amount of antihaemophilic activity in International Units. (One International Unit is the activity equivalent to the average Factor VIII content of 1 ml aliquots of 167 samples of fresh normal plasma, as determined in an international collaborative study.)

Each vial also contains sufficient sodium chloride to make the reconstituted solution approximately isotonic when Water for Injections B.P. is added as directed

1000 iu : 50 ml vial
500 iu : 50 ml vial
250 iu : 30 ml vial

Potency is stated on each vial label.

LEGAL CATEGORY

P.O.M.

REFERENCES

1. Abildgaard. "Current Concepts in the Management of Hemophilia". From "Current Problems in Pediatric Hematology" (Ed. Oski. Jaffe and Miescher), Grune and Stratton, 1975.
2. Abildgaard et al. "Treatment of Hemophilia with Glycine—Precipitated Factor VIII" N. Engl. J. Med., 1966, 275, 471.
3. Bangham, Biggs et al. "A Biological Standard for Measurement of Blood Coagulation Factor VIII Activity". Bull. Wld. Hlth. Org., 1971, 45, 337.
4. Biggs et al. "Factor VIII Concentrates Made in the United Kingdom and the Treatment of Haemophilia based on Studies made During 1969-1972". Brit. J. Haematol., 1974, 27, 391.
5. Brinkhous et al. "A New High-Potency Glycine-Precipitated Antihemophilic Factor (AHF) Concentrate". J. Amer. Med. Ass., 1968, 205, 613.
6. Britton, Harrison and Abildgaard. "Early Treatment of Hemophilic Hemarthroses with Minimal Dose of New Factor VIII Concentrate". J. Pedlat., 1974, 85, 245.
7. Dormandy. "Haemophilia A and B." Prescribers J., 1977, 17, 8.
8. George and Breckenridge. "The use of Factor VIII and Factor IX Concentrates During Surgery". J. Amer. Med. Ass., 1970, 214, 1673.
9. Mazza et al. "Antihemophilic Factor VIII in Hemophilia: Use of Concentrates to Permit Major Surgery". J. Amer. Med. Ass., 1970, 211, 1818.
10. Rizza "Clinical Management of Haemophilia" Br. Med. Bull. 1977, 3, 225-230.

Manufactured by Armour Pharmaceutical Company, U.S.A.
Distributed by

Armour Pharmaceutical Company Limited, Eastbourne, England.

August 1980. PL0231/0044

RECOMMENDED RECONSTITUTION

Reconstitute HIGH POTENCY FACTORATE using the appropriate quantity of Water for Injections B.P. as shown below, using standard aseptic precautions.

1000 iu : 20 ml Water for Injections B.P.
500 iu : 20 ml Water for Injections B.P.
250 iu : 10 ml Water for Injections B.P.

Warm both diluent and HIGH POTENCY FACTORATE to between 20°C and 30°C. Direct diluent down the side of the vial and gently rotate the vial until contents are dissolved. DO NOT SHAKE VIAL. Vigorous shaking will cause frothing and prolong the reconstitution time. Complete solution usually takes approximately 10 minutes. The solution is now ready for administration. If a gel forms on reconstitution, the preparation should not be used. The solution should be used within three hours of reconstitution.

ADMINISTRATION

Standard aseptic techniques should be used at all times.

Intravenous Injection

Plastic disposable syringes are recommended with Factor VIII solution. The ground glass surfaces of all-glass syringes tend to stick with solutions of this type.

1. Attach a filter needle to a sterile disposable syringe. Insert filter needle into stopper of Factor VIII vial; inject air and withdraw the reconstituted solution from the vial.
2. Discard the filter needle and attach suitable intravenous needle.
3. Administer solution by slow intravenous injection, at a rate comfortable to the patient, and not exceeding 2 ml per minute.

Intravenous Infusion

The infusion equipment used should comply with that described in section 3 or 4 of British Standard 2463: 1962, Transfusion Equipment for Medical Use.

1. Prepare solution of HIGH POTENCY FACTORATE as recommended under Reconstitution.
2. Attach suitable infusion set.
3. If more than one vial is to be administered to the same patient the infusion set may be transferred to a second vial.
4. When infusion of HIGH POTENCY FACTORATE is complete, the infusion set may be flushed with sterile isotonic saline to avoid loss of any of the reconstituted solution.
5. After use, discard infusion set, needles and vials together with any unused solution.

DOSAGE

HIGH POTENCY FACTORATE is for Intravenous administration only. As a general rule one unit of Factor VIII activity per kg will increase by 2% the circulating Factor VIII level, and although dosage must be adjusted according to the needs of the patient (weight, severity of haemorrhage, presence of inhibitors) the following general dosages are suggested.

1. **Overt Bleeding**—initially 20 units per kg of body weight followed by 10 units per kg every eight hours for the first 24 hours and the same dose every 12 hours for the next 3 or 4 days. For massive wounds, give until bleeding stops and maintain with 20 units per kg 8-hourly to achieve a minimum Factor VIII level of 40%.
2. **Muscle Haemorrhages**
   (a) Minor haemorrhages in extremities or non-vital areas: 10 units per kg once a day for 2 or 3 days.
   (b) Massive haemorrhages in non-vital areas: 10 units per kg by infusion at 12 hour intervals for 2 days and then once a day for 2 more days.
   (c) Haemorrhages near vital organs (neck, throat, subperitoneal): 20 units per kg initially; then 10 units per kg every 8 hours. After 2 days the dose may be reduced by one-half.
3. **Joint Haemorrhages**—10 units per kg every 8 hours for a day; then twice daily for 1 or 2 days. If aspiration is carried out, 10 units per kg just prior to aspiration with additional infusions of 10 units per kg 8 hours later and again on the following day.
4. **Surgery**—Dosages of 30 to 40 units per kg bodyweight prior to surgery are recommended. After surgery 20 units per kg every 8 hours should be administered. Close laboratory

To the Medical and Pharmaceutical Professions only.

Dried Human Antihaemophilic Fraction B.P. (Sterile) HIGH POTENCY FACTORATE

Armour Pharmaceutical Company Limited
Eastbourne, England

1647-10

AP000589

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
WASHINGTON, D.C.

ESTABLISHMENT LICENSE

FOR THE MANUFACTURE OF
BIOLOGICAL PRODUCTS

This is to certify that Establishment License No. 149 is hereby issued to Armour Pharmaceutical Company, the manufacturer, located at Kankakee, Illinois, through the establishment identified as Armour Pharmaceutical Company, located at Kankakee, Illinois, pursuant to Section 351 of the Public Health Service Act, approved July 1, 1944 (58 Stat. 702, 42 U.S.C. 262), as amended, and the regulations thereunder. The license authorizes the manufacturer to maintain an establishment for the propagation or manufacture and preparation for sale, barter, or exchange in the District of Columbia, or for sending, carrying, or bringing for sale, barter, or exchange from any State or possession into any other State or possession or into any foreign country, or from any foreign country into any State or possession, any virus, therapeutic serum, toxin, antitoxin, vaccine, blood, blood component or derivative, allergenic product, or analogous product, or arsphenamine or its derivatives, for which the manufacturer holds an unsuspended and unrevoked product license issued by the Secretary of Health, Education, and Welfare pursuant to said Act and regulations.

SUPP

Date JAN 05 1979

GRO-C

for Director, Bureau of Biologics
Food and Drug Administration

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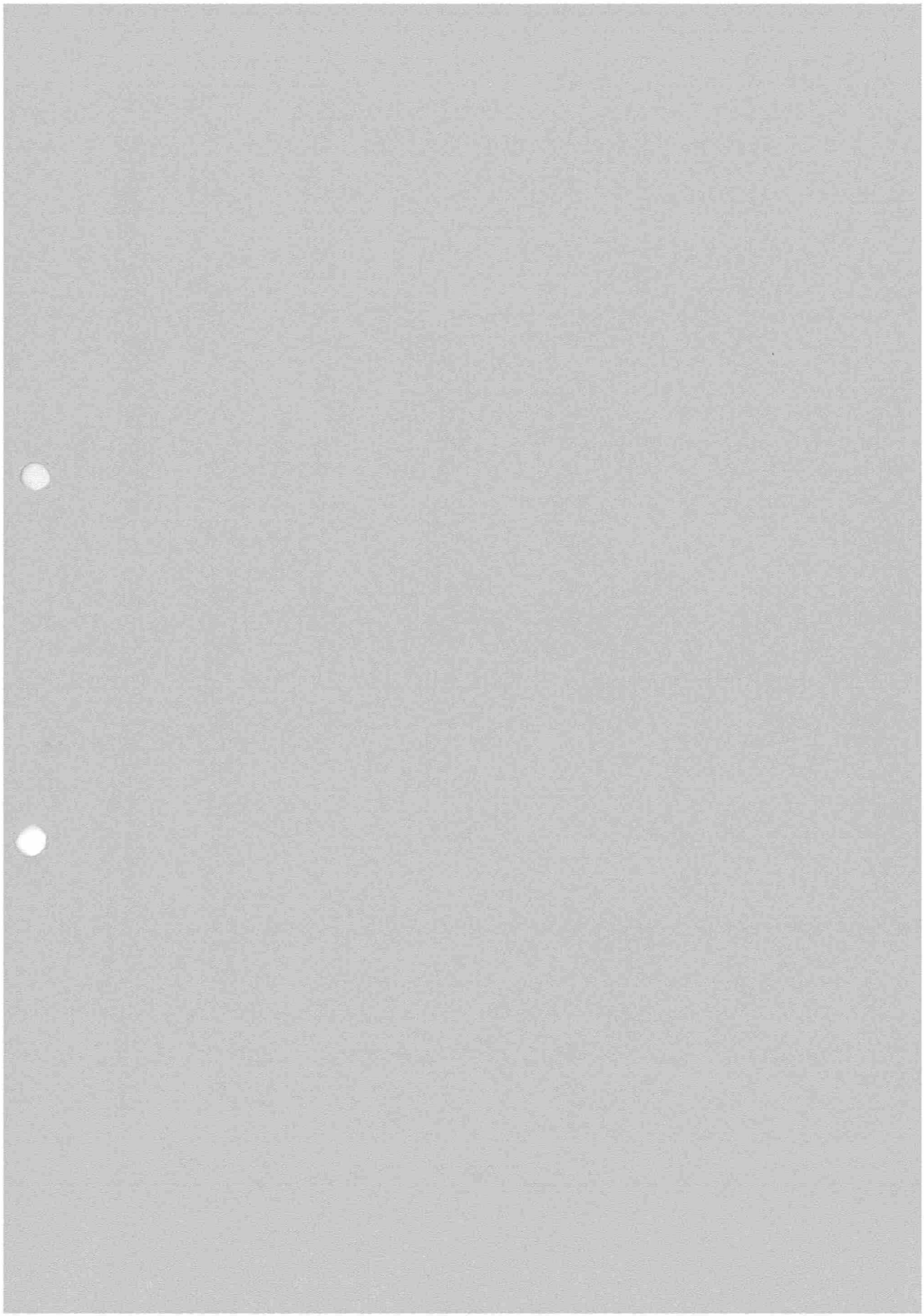

APPLICATION FOR A PRODUCT AUTHORISATION FOR

H I G H P O T E N C Y F A C T O R A T E

PART II CHEMISTRY AND PHARMACY PAGE NO.

CONTENTS

AP000590(A)

PART II CHEMISTRY AND PHARMACY

1. FINISHED PRODUCT

1.1. Description

High Potency Factorate is supplied in 50 or 30 ml Type 1 glass vials containing white to pale yellow lyophilised cake.

1.2. Complete Formula

The formulation of the bulk solution filled into the vials prior to lyophilisation is as follows:

1.2.1. Active

Dried Human Antihaemophilic Fraction BP	..	Approximately 27 iu/ml

1.2.2. Others

Glycine USP	0.2 M
Sodium Citrate USP	0.04M
Sodium Chloride USP	0.04M
Sodium Heparin Injection USP	nmt 1 unit/ml

The lyophilised finished product has the following composition:

Active

Dried Human Antihaemophilic Fraction BP	.. ..	1000, 500 or 250 iu/vial*

Others

Glycine	50mM/litre approx.
Sodium	nmt 200mM/litre
Citrate	nmt 55mM/litre
Heparin	nmt 30 iu/vial

*These potency values are nominal and the actual potency in international units is stated on each vial. Minimal and maximal potencies for these preparations are 80 - 125% of nominal potency in accordance with BP limits.

1.3. Containers

High Potency Factorate is supplied in 50 ml or 30 ml Type 1 clear glass vials with grey butyl rubber lyophilisation stoppers and aluminium seals fitted with brown, non-traumatic flip-top caps.

2. METHOD OF MANUFACTURE

High Potency Factorate is manufactured from fresh-frozen human plasma which, when tested complies with Raw Material Specification 3029 and has been found negative for Hepatitis B surface antigen activity.

A cryoprecipitate is isolated from thawed human plasma and is dissolved at 25° ± 5°C in glycine-saline buffer containing nmt 3 units/ml Sodium Heparin USP. The pH is adjusted with 0.1N Acetic Acid and/or 0.5N sodium hydroxide and filtered. Impurities are adsorbed onto aluminium hydroxide sterile suspension, centrifuged at approximately 15°C and the preparation stabilised with Sodium Citrate USP and Sodium Chloride USP (both pyrogen-free). The solution is cooled to approximately 0°C and cold ethyl alcohol (95%) added to a concentration of approximately 7%. The precipitate is isolated at low temperature and suspended in citrate-saline-glycine buffer. The pH is adjusted to 7.0 ± 0.2 with 0.5M sodium, hydroxide. This solution may be stored at -40°C or colder if required at this stage. Such frozen solutions are thawed at 34 ± 4°C and brought to final volume with buffer. The pH is adjusted to 5.6 ± 0.3 with 0.5 acetic acid at controlled room temperature (15 - 30°C) and the solution is cooled to 8°C ± 5°C for up to 2 hours. The resulting precipitate is separated and the supernatant clarified by membrane filtration and the pH adjusted to 7.2 ± 0.4 with 0.5 M Sodium Hydroxide.

The solution is membrane filtered and finally sterile filtered through bacterial retentive membrane filters (0.8µ down to 0.22µ) before filling into sterile, Type I glass vials. The filled vials are frozen, lyophilised under vacuum and sealed.

A flow chart of the manufacturing procedure is provided overleaf.

FLOW DIAGRAM (cont.)

PHASE J - Sterile Filtration into Sterile Holding Tanks
(Sample submitted for Bulk Sterility Testing)

↓

PHASE K - Filling (Under Constant Positive Pressure)

↓

PHASE L - Lyophilisation (Under vacuum) and Sealing of Vials for Inspection and Storage at 2-8°C or colder.

3. QUALITY CONTROL

3.1. Specification of Constituents

	Specification
Source Plasma (Human) Flash Frozen for Antihaemophilic Factor	3029
Sodium Citrate USP	267
Sodium Chloride USP	271
Glycine USP	753
Sodium Heparin Injection USP	2951, 3403, 3404 or 3407
In-Process Materials	
Sodium Hydroxide USP	USP
Sodium Bicarbonate USP	270
Rehsorptar (F-5000 Aluminium hydroxide gel - sterile)	3232
Glacial Acetic Acid USP	897

3.2. Analytical Methods

Analytical methods used to define the various criteria cited in the above Specifications are provided in numerical sequence in an appendix to this Section.

3.3. In-Process Control

Physical conditions, ie temperature and pH, used during the manufacturing process are critical and consequently sophisticated telemetry has been developed by the Company to ensure that the required conditions prevail throughout the manufacturing process.

The sterile bulk material is tested for sterility in accordance with the in-house method for bulk sterility testing (Method 303) before filling.

3.4. Finished Product Specification

High Potency Factorate products comply with Finished Product Specification Nos. 31 (1000 iu/vial), 101 (500 iu/vial) and 102 (250 iu/vial). Copies of these Specifications are attached.

Analytical methods used in determining these specification limits are supplied in the appendix at the end of this Section.

Full quality control on the finished product is carried out to the Finished Product Specification by Armour Pharmaceutical Company, Kankakee. Responsibility for release of the product based on the results of the analysis supplied with each batch, rests with the Quality Control Manager, at Armour Pharmaceutical Company Ltd., Eastbourne.

3.1. RAW MATERIAL SPECIFICATIONS

Armour Pharmaceutical Company
QUALITY STANDARDS
Kankakee, Illinois

Spec. No. 3029

SPECIFICATION

SOURCE PLASMA (HUMAN), FLASH FROZEN FOR ANTIHEMOPHILIC FACTOR (HUMAN)

DATE:	SUPERSEDES:	PREPARED BY:
3/18/80	2/1/79	B. L. Springer

Description:

SOURCE PLASMA (HUMAN), FLASH FROZEN, FOR ANTIHEMOPHILIC FACTOR (HUMAN), S-3029, is the fluid portion of human blood which has been stabilized against clotting, collected by plasmapheresis from adult humans who have not been hyperimmunized to produce specific antibodies, and intended as source material for manufacture of Anti-hemophilic Factor (Human), Immune Serum Globulin (Human), Plasma Protein Fraction (Human), and Normal Serum Albumin (Human). It is manufactured according to and conforms to all sections of Title 21 of the Code of Federal Regulations, Subchapter F, Parts 600, 601, 606, 607, 610, and Subpart G (640.60 through 640.76) of Subchapter F and is flash frozen as individual units at -70°C. or colder within one hour after separation from red blood cells and within two hours of withdrawal from the donor. The type of anticoagulant contained in the plasma shall be separately agreed upon by authorized representatives of Armour Pharmaceutical Company and the supplier.

Plasma Properties (Specification S-3029):

1. Substantially free from red blood cells.
2. Maximum of 50 mg. Hemoglobin per 100 ml.
3. Total Protein content of not less than 5.5% after processing to remove red blood cells.
4. Free of bacterial or pyrogenic contamination.

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	Spec. No. 3029
SPECIFICATION	SOURCE PLASMA (HUMAN), FLASH FROZEN FOR ANTIHEMOPHILIC FACTOR (HUMAN)

DATE	SUPERSEDES:	PREPARED BY:
3/18/80	2/1/79	B. L. Springer

5. Free of Hepatitis B Surface Antigen as tested on individual units of plasma by Radioimmune Assay or other assay meeting requirements of Title 21 CFR 610.40.

6. Serologically non-reactive for Syphillis.

Labels and Shipping:

1. The label affixed to each immediate container of plasma shall contain all information required in 21 CFR 640.70.

2. The following information shall appear on the label affixed to each carton of plasma.

   a. Addressee - Armour Pharmaceutical Company.

   b. Name and full address of blood establishment.

   c. Proper name of and specification number for material in shipment. "Source Plasma (Human), Flash Frozen, For Antihemophilic Factor (Human), S-3029".

   d. Number and size of containers in carton.

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	Spec. No. 3029
SPECIFICATION	SOURCE PLASMA (HUMAN), FLASH FROZEN FOR ANTIHEMOPHILIC FACTOR (HUMAN)

DATE:	SUPERSEDES:	PREPARED BY:
3/18/80	2/1/79	B. L. Springer

2. (Con't.)

   e. Number of cartons in shipment and the individual number of each carton, e.g., Box #5 of 23 boxes.

   f. The statement, "Store at -20°C. or colder".

3. The following information will be included with each shipment of plasma to Armour Pharmaceutical Company. Records should be contained in an envelope or otherwise adequately bound and placed in one carton of the shipment; that carton should be adequately marked to facilitate its identification when received at Armour Pharmaceutical Company.

   a. The name and full address of the licensed plasmapheresis center at which the plasma was collected.

   b. Identification by number of each plasma unit included in the shipment.

   c. Bleeding dates of all units.

   d. Type of plasma in the shipment.

   e. A statement defining the type of anticoagulant contained in all units in the shipment.

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	Spec. No. 3029
SPECIFICATION	SOURCE PLASMA (HUMAN), FLASH FROZEN FOR ANTIHEMOPHILIC FACTOR (HUMAN)

DATE	SUPERSEDES:	PREPARED BY:
3/18/80	2/1/79	B. L. Springer

3. (Con't.)

f. A statement confirming that all units have been tested and found non-reactive for Hepatitis B Surface Antigen as defined in Title 21, Code of Federal Regulations, §640.67.

g. A statement confirming that all units have been flash frozen at -70°C. or colder within one hour after separation from red blood cells and within two hours of withdrawal from the donor.

NOTE: Forms similar to Attachments A and B may be used.

Storage Conditions:

The plasma must be kept at temperatures of -20°C. or lower.

NOTE: When the freezer storage temperature inadvertantly exceeds -20°C., but not -5°C., for 24 hours or less (eg., as a result of equipment failure or power outage), the plasma in storage will continue to qualify as Source Plasma (Human), Flash Frozen for Antihemophilic Factor (Human), S-3029, provided that:

- continued on next page -

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	Spec. No. 3029
SPECIFICATION	SOURCE PLASMA (HUMAN), FLASH FROZEN FOR ANTIHEMOPHILIC FACTOR (HUMAN)

DATE	SUPERSEDES:	PREPARED BY:
3/18/80	2/1/79	B. L. Springer

NOTE: (Con't.)

a. The source of difficulty is promptly corrected,
b. The temperature is restored to and maintained at the proper level (-20°C. or lower), and,
c. The circumstances involving the incident and the corrective action are formally documented by a responsible individual.

Inspection:

The processing establishment, and all its equipment and records will be available for inspection during regular business hours by designated representatives of Armour Pharmaceutical Company.

Records:

Records of all operations shall be maintained according to the requirements of the Source Plasma (Human) Regulations, but not less than 12 years after manufacture.

General:

1. Failure to comply with any of the foregoing specifications shall be sufficient cause to reject any Plasma delivered to Armour Pharmaceutical Company.

AP000602

Armour Pharmaceutical Company
QUALITY STANDARDS
Kankakee, Illinois

Spec. No. 3029

SOURCE PLASMA (HUMAN), FLASH FROZEN FOR ANTIHEMOPHILIC FACTOR (HUMAN)

SPECIFICATION

DATE:	SUPERSEDES:	PREPARED BY:
3/18/80	2/1/79	B. L. Springer

2. Armour Pharmaceutical Company reserves the right to exclude any and all plasma that may, in its opinion, contribute to processing problems or unsatisfactory final products.

3. Plasma volume is calculated by converting Armour weights using 1.03 as the specific gravity.

4. Plasma bag tare weights used by Armour are as follows:

a. 300 ml. Fenwall bag - 25 grams
b. 800 ml. Fenwall bag - 32 grams
c. 2 liter Fenwall bag - 62 grams
d. 2 liter McGaw bag - 67 grams
e. 800 ml. Cutter bag - 30 grams

ATTACHMENT A

DONOR CENTER ______________________

____

____

Carton Number	Plasma Unit Identity	Date of Bleeding	Plasma Volume	Carton Number	Plasma Unit Identity	Date of Bleeding	Plasma Volume

ATTACHMENT B

HUMAN PLASMA DATA SHEET

(To be submitted with each shipment of plasma sent to Armour Pharmaceutical Company, Kankakee, Illinois)

DONOR CENTER: Name _____

Address _____

TYPE OF PLASMA:

_____ Source Plasma (Human) Flash Frozen for Antihemophilic Factor (Human)
_____ Source Plasma (Human) Flash Frozen for Tetanus Immune Globulin (Human) and Anti-hemophilic Factor (Human)
_____ Source Plasma (Human)
_____ Source Plasma (Human) for Tetanus Immune Globulin (Human)
_____ Source Plasma (Human) Salvaged
_____ Source Plasma (Human) Salvaged for Tetanus Immune Globulin (Human)

Shipment No. _____

Quantity _____ Liters

Date of Shipment _____

This shipment includes plasma collected from _____ through _____ inclusively, and sequentially numbered from _____ through _____.

NTICOAGULANT:

_____ Anticoagulant Sodium Citrate
_____ Anticoagulant Citrate Dextrose
_____ Anticoagulant Citrate Phosphate Dextrose

EPATITIS B SURFACE ANTIGEN

Test Used _____

All units in this shipment have been tested for and found non-reactive for Hepatitis B Surface Antigen.

Yes _____ No _____

Bleed numbers of plasma tested and found reactive for HBSAg from _____ through _____ (use same dates listed above:

_____

_____

Record or dispositon of these units are attached.

AP000605

ATTACHMENT B (CON'T.)

PROCESSING INFORMATION:

All units in this shipment were flash frozen at -70°C. or colder within one hour after separation from red blood cells and within two hours of withdrawal from the donor.

Yes _____ No _____

Explain Exceptions _____

_____

STORAGE CONDITIONS:

Room Temperature _____
Refrigeration _____
Freezer (Below 0°C.) _____
Freezer (-20°C. or colder) _____
Freezer (-40°C. or colder) _____

SIGNATURE _____ TITLE _____

DATE _____

AP000606

Armour Pharmaceutical Company
Quality Control
Kankakee, Illinois

Spec. No. 267

SODIUM CITRATE USP OR REAGENT

SPECIFICATION

Date:	Supersedes:	Prepared by:	Exp. Date:
11/1/73	5/23/56	G. A. Portinga	1 year

DESCRIPTION:

Colorless crystals or a white, crystalline powder. It has a cooling, saline taste.

SAMPLING:

Submit one 100 cc. RWM bottle and one shell vial for reserve sample.
Group I: RWM bottle.

TEST	SPECIFICATION	METHOD
Solubility	O. K.	USP
*Identification	O. K.	USP
Alkalinity	O. K.	USP
*Loss on Drying	10 - 13%	USP
Tartrate	Nil	USP
Heavy Metals	NMt 10 ppm	USP
*Assay	NLT 99.0%; NMT 100.5%	USP

*Test run at Kankakee

Reason for Revision:

THIS SPECIFICATION WAS TRANSFERRED FROM AN APPROVED HALF SHEET. NO FURTHER APPROVALS ARE NECESSARY.

Approved by:

AP000607

ARMOUR000813

ARMO0000092_0027

Armour Pharmaceutical Company
QUALITY STANDARDS
Kankakee, Illinois

Spec. No. 271

SODIUM CHLORIDE U.S.P. - PYROGEN FREE

SPECIFICATION

DATE:	SUPERSEDES:	PREPARED BY:
3/9/77	2/14/75	M. S. Johnson

Description:

Sodium Chloride appears as colorless, cubic crystals or white crystalline powder with a saline taste.

Sampling:

Group I - 1 x 120 g.; Group X - 1 x 5 g. in sterile bottle; Reserve - 1 x 240 g.

TEST	SPECIFICATION	METHOD
Identification	Satisfactory	U.S.P.
Acidity or alkalinity	Maximum of 1.0 ml. of 0.02N NaOH	
	Maximum of 3.12 ml. of 0.02N HCl	U.S.P.
Arsenic	Maximum of 0.0003%	U.S.P.
Barium	The solutions are equally clear after standing for 2 hours	U.S.P.
Heavy metals	Maximum of 0.0005%	U.S.P.
Loss on drying	Maximum of 0.5%	U.S.P.
Iodide or bromide	No violet, orange, or yellow color	U.S.P.
Calcium and magnesium	Maximum of 0.005%	U.S.P.
Sulfate	Maximum of 0.015%	U.S.P.
Sodium ferrocyanide	Nil	U.S.P.
Assay	99.0 - 101.0% (Dry Basis)	U.S.P.
Pyrogen	Satisfactory	U.S.P.
Iron	Maximum of 0.0002%	U.S.P.

Armour Pharmaceutical Company
QUALITY STANDARDS
Kankakee, Illinois

Spec. No. 753

SPECIFICATION

AMINOACETIC ACID, U.S.P. (GLYCINE)

DATE:	SUPERSEDES:	PREPARED BY:
6/13/79	1/14/77	G. A. Portinga

Description:

Aminoacetic Acid occurs as a white, odorless, crystalline powder, possessing a sweetish taste. Its solution is acid to litmus.

Sampling:

Group I - 1 x 20 g.; Group X - 1 x 5 g. sterile sample; Reserve - 1 x 40 g.

TEST	SPECIFICATION	METHOD
Identification	Satisfactory	U.S.P.
Loss on drying	Maximum of 0.2%	U.S.P.
Residue on ignition	Maximum of 0.1%	U.S.P.
Chloride	Maximum of 0.007%	U.S.P.
Sulfate	Maximum of 0.0065%	U.S.P.
Heavy metals	Maximum of 0.002%	U.S.P.
Readily carbonizable substances	Colorless solution	U.S.P.
Hydrolyzable substances	Satisfactory	U.S.P.
Assay	98.5 - 101.5%	U.S.P.
Pyrogen	Satisfactory	208

Armour Pharmaceutical Company
QUALITY STANDARDS
Kankakee, Illinois

Spec. No. 3103

SPECIFICATION

SODIUM HEPARIN INJECTION U.S.P.,
1,000 UNITS/ML., 10 ML. VIAL

DATE:	SUPERSEDES:	PREPARED BY:
9/30/76	5/18/76	G. A. Dunne

Description:

A clear sterile solution of sodium heparin with sodium chloride added to render the solution isotonic. The sodium heparin is derived from porcine intestinal mucosa. It exhibits a potency not less than 90.0 percent and not more than 110.0 percent of the potency stated on the label as expressed in U.S.P. Heparin Units. It meets U.S.P. requirements for injection.

Sampling:

Group I - 3 x 10 ml. vials; Group III - 23 x 10 ml. vials;
Group X - 3 x 10 ml. vials; Reserve - 12 x 10 ml. vials.

TEST	SPECIFICATION	METHOD
Sterility	Satisfactory	U.S.P.
Pyrogen	Satisfactory	U.S.P.
pH	5.0 - 7.5	U.S.P.
Assay	1000 Units/Ml. ± 10%	U.S.P.
Volume in container	Satisfactory	U.S.P.
Benzyl alcohol	1.0 - 1.5% (w/v)	U.S.P.

AP000610

Armour Pharmaceutical Company
QUALITY STANDARDS
Kankakee, Illinois

Spec. No. 3101

SPECIFICATION

SODIUM HEPARIN INJECTION U.S.P.,
5,000 UNITS/ML., 1 ML. VIAL

DATE:	SUPERSEDES:	PREPARED BY:
10/28/76	9/30/76	M. S. Johnson

Description:

A clear sterile solution of sodium heparin with sodium chloride added to render the solution isotonic. The sodium heparin is derived from porcine intestinal mucosa. It exhibits a potency not less than 90.0 percent and not more than 110.0 percent of the potency stated on the label as expressed in U.S.P. Heparin Units. It meets U.S.P. requirements for injection.

Sampling:

Group I - 25 x 1 ml. vials; Group III - 43 x 1 ml. vials;
Group X - 5 x 1 ml. vial; Reserve - 56 x 1 ml. vials.

TEST	SPECIFICATION	METHOD
Sterility	Satisfactory	U.S.P.
Pyrogen	Satisfactory	U.S.P.
pH	5.0 - 7.5	U.S.P.
Assay	5000 Units/Ml. ± 10%	U.S.P.
Volume in container	Satisfactory	U.S.P.
Benzyl alcohol	1.0 - 1.5% (w/v)	U.S.P.

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	Spec. No. 3107
SPECIFICATION	SODIUM HEPARIN INJECTION U.S.P., 10,000 UNITS/ML., 5 ML. VIAL

DATE:	SUPERSEDES:	PREPARED BY:
9/30/76	5/18/76	G. A. Dunne

Description:

A clear sterile solution of sodium heparin with sodium chloride added to render the solution isotonic. The sodium heparin is derived from porcine intestinal mucosa. It exhibits a potency not less than 90.0 percent and not more than 110.0 percent of the potency stated on the label as expressed in U.S.P. Heparin Units. It meets U.S.P. requirements for injection.

Sampling:

Group I - 8 x 5 ml. vials; Group III - 23 x 5 ml. vials;
Group X - 1 x 5 ml. vial; Reserve - 22 x 5 ml. vials.

TEST	SPECIFICATION	METHOD
Sterility	Satisfactory	U.S.P.
Pyrogen	Satisfactory	U.S.P.
pH	5.0 - 7.5	U.S.P.
Assay	10,000 Units/Ml. ± 10%	U.S.P.
Volume in container	Satisfactory	U.S.P.
Benzyl alcohol	1.0 - 1.5% (w/v)	U.S.P.

AP000612

ARMOUR000818

ARMO0000092_0032

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	Spec. No. 270
SPECIFICATION	SODIUM BICARBONATE U.S.P.

DATE:	SUPERSEDES:	PREPARED BY:
3/7/77	12/15/75	M. S. Johnson

Description:

Sodium bicarbonate is a white crystalline powder. Is stable in dry air, but slowly decomposes in moist air. Its solutions, when freshly prepared with cold water, without shaking, are alkaline to litmus. The alkalinity increases as the solutions stand, are agitated, or are heated.

Sampling:

Group I - 1 x 20 g.; Group X - 1 x 2 g. in sterile bottle; Reserve - 1 x 40 g.

TEST	SPECIFICATION	METHOD
Identification	Satisfactory	U.S.P.
Loss on drying	Maximum of 0.25%	U.S.P.
Insoluble substances	Complete and clear solution	U.S.P.
Normal carbonate	Solution does not assume more than a faint pink color immediately	U.S.P.
Ammonia	No odor of ammonia is evolved	U.S.P.
Arsenic	Maximum of 0.0003%	U.S.P.
Heavy metals	Maximum of 0.0005%	U.S.P.
Assay	99.0 - 100.5%	U.S.P.
Pyrogen	Satisfactory	U.S.P.

AP000613

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	Spec. No. 3232
SPECIFICATION	REHSORPTAR, 1 LITER (ALUMINUM HYDROXIDE, STERILE SUSPENSION)

DATE:	SUPERSEDES:	PREPARED BY:
5/6/77	2/20/74	M. S. Johnson

Description:

Rehsorptar, 1 Liter (Aluminum Hydroxide, Sterile Suspension) is a sterile, opaque, white, viscous, thixotropic gel.

Sampling:

Group I - 1 partial bottle; Group III - 20 partial fill 1 liter bottles; Reserve - 2 x 200 ml. vials.

TEST	SPECIFICATION	METHOD
Aluminum oxide	1.8 - 2.2%	813
Protein binding capacity	Minimum of 1 mg. protein per mg. Aluminum Oxide	854
Specific gravity	0.900 - 1.100	83B
Sterility	Satisfactory	303

Armour Pharmaceutical Company
QUALITY STANDARDS
Kankakee, Illinois

Spec. No. 897

GLACIAL ACETIC ACID REAGENT GRADE, U.S.P.

SPECIFICATION

DATE:	SUPERSEDES:	PREPARED BY:
12/7/76	7/28/75	M. S. Johnson

Description:

Glacial Acetic Acid Reagent Grade, U.S.P. is a clear colorless liquid having a characteristic pungent odor.

Sampling:

Group I - 2 x 500 ml. in clean, dry, glass stoppered flask.
Reserve - No reserve.

TEST	SPECIFICATION	METHOD
Assay (Freezing Point)	Not below 16.0°C.	A.C.S.*
Color (APHA)	Not more than 10	A.C.S.
Dilution test	To pass test	A.C.S.
Residue after evaporation	Maximum of 0.001%	A.C.S.
Acetic anhydride	Maximum of 0.01%	A.C.S.
Chloride (Cl)	Maximum of 1 ppm	A.C.S.
Sulfate (SO_4)	Maximum of 1 ppm	A.C.S.
Heavy metals (as Pb)	Maximum of 0.5 ppm	A.C.S.
Iron (Fe)	Maximum of 0.2 ppm	A.C.S.
Substances reducing dichromate	To pass test	A.C.S.
Substances reducing permanganate	To pass test	A.C.S.
Suitability for non-aqueous titrations	To pass test	A.C.S.
Sensitivity	To pass test	U.S.P.(Reagent)

*U.S.P. specifies A.C.S. test methods.

3.4. FINISHED PRODUCT SPECIFICATIONS

ARMOUR PHARMACEUTICAL COMPANY LIMITED
EASTBOURNE ENGLAND

QUALITY CONTROL DEPARTMENT

SPECIFICATION SHEET NO. 31

Amended September 1980
(Sheet 1 of 2)

HIGH POTENCY FACTORATE
(Nominal 1000 i.u./vial)
(Dried Human Antihaemophilic Fraction (Sterile) B.P.)

Description:	A white to pale yellow lyophilised cake in a 50 ml vial closed with a brown non-traumatic flip-cap.
Sampling:	Ten pre bulk-shipment vials supplied by Q.C. Department, A.P.C., Kankakee. No samples taken of bulk delivery.

TEST	SPECIFICATION	METHOD
Mammalian Protein	Human positive Bovine and porcine negative	351/K
Potency	Not less than 800 i.u./vial (not less than 26.5 i.u./ml when reconstituted with 30 ml Water for Injections B.P.)	B.P.
Heparin	Not more than 30 i.u. per vial	1073/K
Total Protein	Not more than 600 mg per vial (not more than 20 g/litre reconstituted)	993/K
Fibrinogen	Not more than 480 mg per vial (not more than 16 g/litre reconstituted)	1344/K
Aluminium	Not more than 180 µg per vial	995/K
Moisture	Not more than 2% w/w	43-D(K)
Freedom from abnormal toxicity (a) Mouse test (b) Guinea Pig test (Injection n.l.t. 538 i.u./kg of body weight)	 Passes Test Passes Test	963/K
Pyrogens (10 i.u./ kg body weight)	Passes Test	208/K
Sterility	Passes Test	303/K

...../2.

QUALITY CONTROL DEPARTMENT — SPECIFICATION SHEET NO. 31

Amended September 1980
(Sheet 2 of 2)

Solution Time	Not more than 30 minutes, typically less than 10 minutes	1343/K
pH	6.8 - 7.4 (when reconstituted with 30 ml of Water for Injections B.P.)	53/K
Isoagglutinins	Not more than 1:256 without predilution and typically less than 1:64 when tested against Anti-A and Anti-B.	386/K
Sodium	Not more than 200 mM per litre (when reconstituted with 30 ml of Water for Injections B.P.)	1301/K
Citrate	Not more than 55 mM per litre (when reconstituted with 30 ml of Water for Injections B.P.)	1402/K
Hepatitis B_s Antigen	Negative	379/K or 1410/K

Approved by:		Date
Technical Affairs Manager	GRO-C	October 7th 1980
Regulatory Affairs Manager		October 7th, 1980.
Research & Development Manager		October 8th 1980
Product Manager		October 23rd 1980
Manufacturing Manager		
Chief Analyst	GRO-C	24th October 1980
Quality Control Manager		30th October 1980

AP000618

ARMOUR000824

ARMO0000092_0038

ARMOUR PHARMACEUTICAL COMPANY LIMITED
EASTBOURNE ENGLAND

QUALITY CONTROL DEPARTMENT

SPECIFICATION SHEET NO. 101

Amended September 1980
(Sheet 1 of 2)

HIGH POTENCY FACTORATE
(Nominal 250 i.u./vial)

(Dried Human Antihaemophilic Fraction (Sterile) B.P.)

Description:	A white to pale yellow lyophilised cake in a 30 ml vial closed with a brown non-traumatic flip-cap.	
Sampling:	Ten pre bulk-shipment vials supplied by Q.C. Department, A.P.C., Kankakee. No samples taken of bulk delivery.	
TEST	SPECIFICATION	METHOD
Mammalian Protein	Human positive Bovine and porcine negative	351/K
Potency	Not less than 200 i.u./vial (not less than 20 i.u. per ml when reconstituted in 10 ml Water for Injections B.P.)	B.P.
Heparin	Not more than 10 i.u. per vial	1073/K
Total Protein	Not more than 150 mg per vial (not more than 15 g/litre reconstituted)	993/K
Fibrinogen	Not more than 120 mg per vial (not more than 12 g/litre reconstituted)	1344/K
Aluminium	Not more than 50 µg per vial	995/K
Moisture	Not more than 2% w/w	43-0(K)
Freedom from abnormal toxicity (a) Mouse test (b) Guinea Pig test (Injection n.l.t. 538 i.u./kg of body weight)	 Passes Test Passes Test	963/K
Pyrogens (10 i.u./kg body weight)	Passes Test	208/K
Sterility	Passes Test	303/K

QUALITY CONTROL DEPARTMENT

SPECIFICATION SHEET NO. 101

Amended September 1980
(Page 2 of 2)

Solution Time	Not more than 30 minutes, typically less than 10 minutes	1343/K
pH	6.8 - 7.4 (when reconstituted with 10 ml of Water for Injections B.P.)	53/K
Isoagglutinins	Not more than 1:256 without predilution and typically less than 1:64 when tested against Anti-A and Anti-B	386/K
Sodium	Not more than 200 mM per litre (when reconstituted with 10 ml of Water for Injections B.P.)	1301/K
Citrate	Not more than 55 mM per litre (when reconstituted with 10 ml of Water for Injections B.P.)	1402/K
Hepatitis B_s Antigen	Negative	379/K or 1410/K

Approved by:

Technical Affairs Manager	October 7th, 1980
Regulatory Affairs Manager	October 7th, 1980
Research & Development Manager	Octob 8th 1980
Production Manager	23rd October 1980
Chief Analyst	24th October 1980
Quality Control Manager	30th October 1980

GRO-C

AP000620

ARMOUR000826

ARMO0000092_0040

ARMOUR PHARMACEUTICAL COMPANY LIMITED
EASTBOURNE ENGLAND

QUALITY CONTROL DEPARTMENT

SPECIFICATION SHEET NO. 102

Amended September 1980
(Sheet 1 of 2)

HIGH POTENCY FACTORATE
(Nominal 500 i.u./vial)

(Dried Human Antihaemophilic Fraction (Sterile) B.P.)

Description:	A white to pale yellow lyophilised cake in a 50 ml vial closed with a brown non-traumatic flip-cap.
Sampling:	Ten pre bulk-shipment vials supplied by Q.C. Department, A.P.C., Kankakee. No samples taken of bulk delivery.

TEST	SPECIFICATION	METHOD
Mammalian Protein	Human positive Bovine and porcine negative	351/K
Potency	Not less than 400 i.u./vial (not less than 20 i.u. per ml when reconstituted in 20 ml Water for Injections B.P.)	B.P.
Heparin	Not more than 20 i.u. per vial	1073/K
Total Protein	Not more than 300 mg per vial (not more than 15 g/litre reconstituted)	993/K
Fibrinogen	Not more than 240 mg per vial (not more than 12 g/litre reconstituted)	1344/K
Aluminium	Not more than 100 µg per vial	995/K
Moisture	Not more than 2% w/w	43-0(K)
Freedom from abnormal toxicity (a) Mouse test (b) Guinea Pig test (Injection n.l.t. 538 i.u./kg of body weight)	Passes Test Passes Test	963/K
Pyrogens (10 i.u./ kg body weight)	Passes Test	208/K
Sterility	Passes Test	303/K

AP000621

...../2.

QUALITY CONTROL DEPARTMENT

SPECIFICATION SHEET NO. 102

Amended September 1980
(Sheet 2 of 2)

Solution Time	Not more than 30 minutes, typically less than 10 minutes	1343/K
pH	6.8 - 7.4 (when reconstituted with 20 ml of Water for Injections B.P.)	53/K
Isoagglutinins	Not more than 1:256 without pre-dilution and typically less than 1:64 when tested against Anti-A and Anti-B	386/K
Sodium	Not more than 200 mM per litre (when reconstituted with 20 ml of Water for Injections B.P.)	1301/K
Citrate	Not more than 55 mM per litre (when reconstituted with 20 ml of Water for Injections B.P.)	1402/K
Hepatitis B_s Antigen	Negative	379/K or 1410/K

Approved by:		Date
Technical Affairs Manager	GRO-C	October 7th 1980
Regulatory Affairs Manager		October 7th 1980
Research & Development Manager		October 8th 1980
Production Manager		23rd October 1980
Chief Analyst		24th October 1980
Quality Control Manager		30th October 1980

AP000622

ARMOUR000828

ARMO0000092_0042

4. DEVELOPMENT PHARMACEUTICS

The use of Antihaemophilic Fraction Concentrates in the treatment of classical haemophilia A is well established and Factorate, a product similar to High Potency Factorate, is already Licensed for sale in Eire under Product Authorisation Number PA 10/6/1. The high potency product has been developed as the result of further purification procedures which have resulted in an overall increase in the specific activity of the material, coupled with a reduction in the equivalent levels of protein (notably fibrinogen) and heparin present in the finished product. The end result is a product with greater or similar activity with lower risk of compromise to the circulation through excessive levels of protein.

Batch analysis data for three production batches is attached on the following pages.

LOT K852032

TEST	SPECIFICATION*	ASSAY DATA
AHF Potency [a]	NLT 30 AHF U/Recon. ml and NLT 900 AHF U/Vial	40.6 U/ml 1218 U/Vial
AHF Potency Recon. Stability 3 hrs at Cont. Rm. Temp.[a]	NLT 80% of 0 hour	1119 U/Vial 91.8% of 0 hr
Heparin Assay [a]	NMT 1 U/Recon. ml NMT 30 U/Vial	0.4 U/ml 12 U/Vial
Total Protein[a]	For Calculation	44.3 mg/ml or 1329 mg/Vial
Clottable Protein[a]		28.9 mg/ml or 867 mg/Vial
Specific Activity	NLT 0.5 AHF U/mg protein or NMT 2.0 mg protein/AHF U	0.916 U/mg 1.091 mg/U
Aluminium [a]	LT 0.0002 mg/Recon. ml and LT 0.006 mg/Vial	LT 0.001 mg/25 ml
Moisture	NMT 2% w/w	0.08%
pH[a]		7.23
Identity	Human - Positive Bovine - Negative Porcine - Negative Ovine - Negative	Passes
Safety [a]	Passes	Passes
Sterility	Passes U.S.P.	Passes
Pyrogens [a] (40 AHF U/kg)	Passes U.S.P.	Passes (.5/0/0)
Solution Time[a]	NMT 30'	26'
Isoagglutinins[a] (10 AHF U/ml)	NMT 1:256	Passes
Hepatitis B Surface Antigen (HBsAg) [a]	Negative	Negative
Appearance of Cake	White to Nearly White	White
Particulate Matter		Thres. 10 µ 1154/ml Thres. 25 µ 551/ml

NOTE: a - Reconstituted with 30 ml Sterile Water for Injection U.S.P.

LOT K852031

TEST	SPECIFICATION*	ASSAY DATA
AHF Potency [a]	NLT 30 AHF U/Recon. ml and NLT 900 AHF U/Vial	38.8 U/ml 1164 U/Vial
AHF Potency Recon. Stability 3 hrs at Cont. Rm. Temp.[a]	NLT 80% of 0 Hour	1203 U/Vial 103.4% of 0 hr
Heparin Assay [a]	NMT 1 U/Recon. ml NMT 30 U/Vial	0.4 U/ml 12 U/vial
Total Protein[a]	For Calculation	46.3 mg/ml or 1389 mg/Vial
Clottable Protein[a]		32 mg/ml or 960 mg/Vial
Specific Activity	NLT 0.5 AHF U/ml protein or NMT 2.0 mg protein/AHF U	0.838 U/mg 1.193 mg/U
Aluminium [a]	LT 0.0002 mg/Recon. ml and LT 0.006 mg/Vial	LT 0.001 mg/25 ml
Moisture	NMT 2% w/w	0.03%
pH[a]		7.15
Identity	Human - Positive Bovine - Negative Porcine - Negative Ovine - Negative	Passes
Safety [a]	Passes	Passes
Sterility	Passes U.S.P.	Passes
Pyrogens [a] (40 AHF U/kg)	Passes U.S.P.	Passes (.1/.5/.2)
Solution Time[a]	NMT 30'	14'
Isoagglutinins [a] (10 AHF U/ml)	NMT 1:256	Passes
Hepatitis B Surface Antigen (HBsAg) [a]	Negative	Negative
Appearance of Cake	White to Nearly White	White
Particulate Matter		Thres. 10µ 1010/ml Thres. 25µ 422/ml

NOTE: a - Reconstituted with 30 ml Sterile Water for Injection U.S.P.

AP000625

ARMOUR000831

ARMO0000092_0045

BATCH ANALYSIS RESULTS

LOT K852030

TEST	SPECIFICATION*	ASSAY DATA
AHF Potency [a]	NLT 30 AHF U/Recon. ml and NLT 900 AHF U/Vial	43.8 U/ml 1314 U/Vial
AHF Potency Recon. Stability 3 hrs at Cont. Rm. Temp.[a]	NLT 80% of 0 Hour	1278 U/vial 97.3% of 0 hr
Heparin Assay [a]	NMT 1U/Recon. ml NMT 30 U/Vial	0.4 U/ml 12 U/Vial
Total Protein[a]	For Calculation	45.4 mg/ml or 1362 mg/Vial
Clottable Protein[a]		39.5 mg/ml or 1185 mg/Vial
Specific Activity	NLT 0.5 AHF U/mg protein or NMT 2.0 mg protein/AHF U	0.965 U/mg 1.036 mg/Vial
Aluminium [a]	LT 0.0002 mg/Recon. ml and LT 0.006 mg/Vial	LT 0.001 mg/25 ml
Moisture	NMT 2% w/w	0.04%
pH[a]		7.07
Identity	Human - Positive Bovine - Negative Porcine - Negative Ovine - Negative	Passes
Safety [a]	Passes	Passes
Sterility	Passes U.S.P.	Passes
Pyrogens [a] (40 AHF U/kg)	Passes U.S.P.	Passes (.2/1.1/.6/.3/.5/.4/.4/.1)
Solution Time[a]	NMT 30'	14'
Isoagglutinins [a] (10 AHF U/ml)	NMT 1:256	Passes
Hepatitis B Surface Antigen (HBsAg) [a]	Negative	Negative
Appearance of Cake	White to Nearly White	White
Particulate Matter		Thres. 10µ 1106/ml Thres. 25µ 418/ml

NOTE: a - Reconstituted with 30 ml Sterile Water for Injection U.S.P.

AP000626

ARMOUR000832

ARMO0000092_0046

5. STABILITY

The proposed shelf-life is two years when stored at refrigerated temperature (less than 8°C), protected from light. The product may be stored for a period of up to six months at room temperature (less than 25°C) within the shelf-life of the product.

ARMOUR PHARMACEUTICAL COMPANY LIMITED EASTBOURNE ENGLAND	Pharmaceutical Document	1
Product: HIGH POTENCY FACTORATE (GENERATION IIB)	STABILITY REPORT	46
	Number 2	Date January 1981
	Replaces	Report No. 1 dated August 1980

BATCHES EXAMINED

Batch No.	Date of Manufacture
S 29212	1978
T 31803	1979
T 34603	1979

COMMENTS ON BATCHES

The three batches under examination are all commercial production lots. The batches are all of the 1000 iu/vial presentation but in view of the fact that the 500 iu/vial and 250 iu/vial presentations are smaller fills of the same material in the same type of container and closure system, the data given in this report are deemed applicable to the lower strength presentations.

CONDITIONS OF STORAGE

Samples of each batch have been stored at temperatures of 2-8°C and 15-30°C (Controlled Room Temperature) and 37°C for the storage periods indicated in the 'Results' section.

CONTAINERS

All batches were stored in the container foreseen for marketing, ie 50 ml Type 1 glass vials fitted with Tompkins 20 mm butyl rubber lyophilisation stopper and one piece aluminium seal.

RESULTS

The results are shown in the tables overleaf. Batches were tested against Armour Pharmaceutical Company, Kankakee Specification equivalent to the Armour Pharmaceutical Company Limited, Eastbourne Specification No. 31.

ARMOUR PHARMACEUTICAL COMPANY LIMITED EASTBOURNE ENGLAND	Pharmaceutical Document 2
Product: HIGH POTENCY FACTORATE (GENERATION IIB)	STABILITY REPORT 47
	Number 2 / Date January 1981
	Replaces Report No. 1 dated August 1980

(i) Potency

BATCH NO.	TEMPERATURE OF STORAGE °C	TESTING INTERVAL	POTENCY IN UNITS/VIAL					
			INITIAL	3 MONTHS	6 MONTHS	12 MONTHS	18 MONTHS	24 MONTHS
S29212	2-8	A	1112	975	960	1038	970	920
	15-30	A	1113	915	945	1147	965	915
T31803	2-8	A	1083	1005	1095	1410	1010	950
	15-30	A	1083	1035	1185	1230	910	975
T34803	2-8	A	933	945	930	960	915	895
	15-30	A	933	961	853	1020	945	875

A = Assay of vial contents

Analytical Method

The methodology used to determine the potency of the vial is Armour Pharmaceutical Company, Kankakee, Method No. 365.

(ii) Solution Time

BATCH NO.	TEMPERATURE OF STORAGE °C	SOLUTION TIME IN MINUTES/VIAL				
		INITIAL	3 MONTHS	6 MONTHS	12 MONTHS	18 MONTHS
S 29212	2-8	3	7, 10	9	10, 7	5, 7
	15-30	-	10, 13	12	10, 6	5, 3
	37	-	15, 18			
T 31803	2-8	7	8	10, 8	9	5
	15-30	7	8, 7	6, 7	5	3, 3
	37	7	10			
T 34603	2-8	9, 9, 10	9, 9	15, 8	5	2, 2
	15-30	9, 9, 10	11, 10	11	4	4, 3
	37	9, 9, 10	13, 8			

Analytical Method

The methodology used to determine solution time for the vials is Armour Pharmaceutical Company, Kankakee Method 1343.

(iii) Moisture

BATCH NO.	TEMPERATURE OF STORAGE °C	PERCENTAGE MOISTURE		
		INITIAL	6 MONTHS	19 MONTHS
S 29212	2-8	0.28	0.15	
T 31803	2-8	0.0	0.42	0.05
T 34503	2-8	0.24	0.31	

Analytical Method

The moisture content of the lyophilised vial content was determined using Armour Pharmaceutical Company, Kankakee Method 43D.

DISCUSSION OF RESULTS

No undue or unusual effects have been noted in the stability programme and the samples tested met the appropriate specification requirements.

CONCLUSIONS

Based on the stability results obtained with the preparation and with reference to the proven stability of Factorate (see separate report) the product is sufficiently stable to be distributed in the packs listed below and with instructions regarding storage and shelf-life as specified below:

ARMOUR PHARMACEUTICAL COMPANY LIMITED EASTBOURNE ENGLAND	Pharmaceutical Document 5	
Product:	STABILITY REPORT 50	
HIGH POTENCY FACTORATE (GENERATION IIB)	Number 2	Date January 1981
	Replaces	Report No. 1 dated August 1980

Packs: Type 1 glass container fitted with butyl rubber lyophilisation stopper and one piece aluminium seal.

SPECIAL INSTRUCTIONS ON THE PACKAGING MATERIAL

Storage:

High Potency Factorate should be stored at a temperature below 8°C and protected from light. The solution should be used within three hours of reconstitution.

Reconstitution:

Reconstitute High Potency Factorate using the appropriate quantity of Water for Injections B.P. as shown below using standard aseptic precautions

1000 iu : 30 ml Water for Injections B.P.
500 iu : 20 ml Water for Injections B.P.
250 iu : 10 ml Water for Injections B.P.

Warm to 20°C - 30°C before reconstitution with the Water for Injections B.P. Gentle mixing should be employed to avoid frothing.

Validity:

24 months when stored at a temperature below 8°C protected from light.

FLOW DIAGRAM

Manufacturing Process for Antihaemophilic Factor (Human), (High Potency)

PHASE A - Collection and Storage of Human Plasma

↓

PHASE B - Isolation of Cryoprecipitate

a) Thawing at $0^{\circ}C \pm 2^{\circ}C$
b) Centrifugation at $1^{\circ}C \pm 2^{\circ}C$ ⟶ Cryo-Poor Plasma Supernatant

↓

PHASE C - Dissolution of Cryoprecipitate in Glycine-Saline Buffer Containing Heparin

a) pH adjustment
b) Centrifugation/Filtration ⟶ Precipitate discarded
c) pH adjustment

↓

PHASE D - Aluminium Hydroxide Adsorption (Sterile 2% Suspension Added)

a) Storing at $15^{\circ}C \pm 5^{\circ}C$
b) Centrifugation and Filtration ⟶ Precipitate discarded

↓

PHASE E - Stabilisation and Alcohol Precipitation

a) Sodium Citrate, Sodium Chloride additions
b) Addition of Ethanol at $0^{\circ}C \pm 2^{\circ}C$

↓

PHASE F - Isolation of Precipitate by Centrifugation ⟶ Discarded Supernatant at $0^{\circ}C \pm 3^{\circ}C$

↓

PHASE G - Resuspension of Precipitate in Citrate-Saline-Glycine Buffer

a) pH adjusted 7.0 ± 0.2 (below $-40^{\circ}C$)

↓

PHASE H - pH adjustment followed by cooling and filtration through membrane filter.

a) pH adjustment to 5.6 ± 0.3 (at $15\text{-}30^{\circ}C$)
b) Cool solution to $8^{\circ}C \pm 5^{\circ}C$ ⟶ Precipitate discarded
c) pH clarified solution adjusted to 7.2 ± 0.4 with 0.5 M Sodium Hydroxide

↓

PHASE I - Clarification through a Membrane Filtration Assembly

↓

(see next page)

AP000593

6. CONTAINERS

High Potency Factorate is supplied in 50 ml or 30 ml Type I clear glass vials with 20 mm neck finish. The closure is a grey butyl rubber lyophilisation stopper fitted with an aluminium seal and brown plastic, non-traumatic flip-top cap.

APPENDIX 1 - NUMERICAL INDEX OF ANALYTICAL METHODS

Armour Pharmaceutical Company
QUALITY STANDARDS
Kankakee, Illinois

APC METHOD NO. 43D

ANALYTICAL METHOD

LOSS OF WEIGHT ON DRYING

DATE:	SUPERSEDES:	PREPARED BY:
9/26/79	New	A. K. Roop

TEST SUMMARY

A weighed sample is exposed to conditions suited for removing volatiles for a specified length of time. The sample is re-weighed and the loss in weight is calculated as percent of the total weight of the sample.

COMMENTS

a. Safety Precautions: Exercise extreme care when handling and disposing of phosphorus pentoxide or sulfuric acid.

b. General Precautions: The desiccators should be charged with an active desiccant. After drying, all weighings must be performed rapidly to minimize moisture adsorption by the dried sample.

MATERIALS FOR TESTING

A. Apparatus

1. Desiccator charged with active phosphorus pentoxide or sulfuric acid.
2. Vacuum pump.
3. McLeod vacuum gauge.

B. Chemicals

1. Phosphorus Pentoxide.
2. Sulfuric Acid.

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD No. 43D
ANALYTICAL METHOD	LOSS OF WEIGHT ON DRYING

TEST PROCEDURE

A. Preparation of Moisture Pans or Weighing Bottles

Prepare the moisture pans or weighing bottles by placing them for 30 minutes under the same conditions to be employed in the determination. After 30 minutes, accurately weigh the container. This is the tare or empty weight.

B. Procedure

Reduce the sample to a fine powder. Uniformly distribute 0.5-1.0 g. of the sample material in the moisture container and accurately weigh to determine the gross weight or container plus undried sample weight. If possible, the exposed surface area of the powdered sample should not be less than 18 square centimeters.

Place the container in a desiccator containing fresh phosphorus pentoxide or concentrated sulfuric acid for a period of 12-24 hours, under a pressure of not more than 500 microns* measured with a McLeod vacuum gauge, or equivalent. Maintain the temperature at 20-25°C. (room temperature). After drying, remove the container and weigh immediately. This weight is the weight of the container plus dried sample.

*To obtain this pressure, a dry ice-acetone trap is necessary between the desiccator and the pump.

INTERPRETATION OF RESULTS

(Container + undried sample weight) - (tare weight) = Weight of undried sample.

(Container + undried sample) - (Container + dried sample weight) = Loss of weight on drying

Then: $\frac{\text{Loss of weight on drying}}{\text{Weight of undried sample}} \times 100 =$ % of sample wt. lost on drying

REFERENCE

Armour Method No. 43D, dated 7/1/76

8/20/79
dw

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD NO. 53
ANALYTICAL METHOD	DETERMINATION OF pH

DATE:	SUPERSEDES:	PREPARED BY:
11/11/80	New	A. K. Roop

TEST SUMMARY

The pH of a sample is determined on its solution using a pH meter fitted with glass and calomel electrodes. Measurements are made at ambient temperature unless otherwise specified.

COMMENTS

a. Safety Precautions: General laboratory precautions prevail.

b. General Precautions: Standardize the pH meter at least once every 4 hours. For the most accurate results, the temperature of the standardizing buffers and sample solution should be equal.

MATERIALS FOR TESTING

1. A pH meter capable of repeating measurements of pH to within ± 0.02 pH units, and millivolts to within ± 1 millivolt.

2. Standardizing Buffer Solutions: Commonly used prepared buffers are pH=4, pH=7, pH=10. For buffers of other pH values, consult the section in the U.S.P. entitled "Buffer Solutions for pH Standardization".

TEST PROCEDURE

The analyst is advised to read the manufacturer's operation instruction manual for the particular instrument being used and the section in the U.S.P. concerning pH.

- continued on next page -

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD No. 53
-ANALYTICAL METHOD	DETERMINATION OF pH

TEST PROCEDURE (Con't.)

The following paragraph has been copied from U.S.P. XX and describes the proper procedure for standardizing and measuring the pH of a sample;

> To standardize the pH meter, select two Buffer Solutions for Standardization whose difference in pH does not exceed 4 units and such that the expected pH of the material under test falls between them. Fill the cell with one of the Buffer Solutions for Standardization at the temperature at which the test material is to be measured. Set the "temperature" control at the temperature of the solution, and adjust the calibration control to make the observed pH value identical with that tabulated. Rinse the electrodes and cell with several portions of the second Buffer Solution for Standardization, then fill the cell with it, at the same temperature as the material to be measured. The pH of the second buffer solution is within ± 0.07 pH unit of the tabulated value. If a larger deviation is noted, examine the electrodes and, if they are faulty, replace them. Adjust the "slope" or "temperature" control to make the observed pH value identical with that tabulated. Repeat the standardization until both Buffer Solutions for Standardization give observed pH values within 0.02 pH unit of the tabulated value without further adjustment of the controls. When the system is functioning satisfactorily, rinse the electrodes and cell several times with a few portions of the test material, fill the cell with the test material, and read the pH value. Use carbon dioxide-free water (see Water, in the section, Reagents, Indicators, and Solutions) for solution or dilution of test material in pH determinations.

INTERPRETATION OF RESULTS

Report the observed pH of the sample solution and if dilution of the original sample was made, state the degree of dilution, such as, 1:10, etc.

11/5/80
dw

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Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD NO. 83
ANALYTICAL METHOD	SPECIFIC GRAVITY

DATE:	SUPERSEDES:	PREPARED BY:
1/3/80	New	L. Cotter

TEST SUMMARY

The specific gravity of a liquid is the quotient obtained by dividing the weight of the substance contained in a vessel by the weight of water contained in the same vessel at the specified temperature.

COMMENTS

a. Safety Precautions: Follow general laboratory safety conditions.

b. General Precautions: The specific gravity bottle and side arm cap should be absolutely clean and dry before use.

MATERIALS FOR TESTING

1. Specific gravity bottle (Pycnometer)
2. Analytical balance
3. Distilled water
4. Lint free cloth

TEST PROCEDURE

Calibrate the bottle using distilled water as a standard by first weighing empty with the thermometer and side arm cap in place. The average of five weighings should be considered as the weight of the empty specific gravity bottle.

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD No. 83
ANALYTICAL METHOD	SPECIFIC GRAVITY

TEST PROCEDURE (Con't.)

The bottle is then filled with fresh distilled water at a temperature of approximately 15-16°C. Insert the thermometer in such a way as to prevent the formation of any large bubbles around the stem and to force excess water from the side arm. Using a lint free cloth, gently wipe the bottle dry. When the thermometer registers exactly 20°C., or the temperature specified, wipe the excess water from the tip of the side arm and place the cap in position. Weigh the filled bottle, recording the weight to the fourth decimal place. The process of filling and weighing the bottle should be repeated five times with the average of these weights considered as the filled weight of the bottle.

When determining the specific gravity of a solution, only one weight determination is necessary.

INTERPRETATION OF RESULTS

$$\frac{\text{Weight of sample filled bottle (grams) - Weight of empty bottle (grams)}}{\text{Weight of distilled water filled bottle (grams) - weight of empty bottle (grams)}}$$

= Specific gravity of sample at specified temperature

REFERENCE

Armour Method 83, dated 4/12/77.

11/13/79
dw

AP000640

ADI-6600

Armour Pharmaceutical Company QUALITY STANDARDS Kenkakee, Illinois	PYROGEN TEST	APC METHOD NO. 208
ANALYTICAL METHOD		

DATE:	SUPERSEDES:	PREPARED BY:
11/23/77	New	L. Cotter

TEST SUMMARY

The pyrogen test is designed to limit to an acceptable level the risks of febrile reaction in the patient due to the administration, by injection, of the product concerned. The dose specified for the test is related to that generally given to the patient, but for practical reasons, it does not exceed 10 ml. per kg. of body weight of the test animal, injected in a brief period of time.

COMMENTS

Safety Precautions - General laboratory safety conditions prevail. Care should be exercised in the handling of the animals and cleaning of the equipment.

General Precautions - None.

MATERIALS FOR TESTING

Apparatus - Render the syringes, needles, and glassware free from pyrogens by heating at 121°C. for not less than 30 minutes or by any other suitable method. Just prior to injecting it, warm the product to be tested to approximately 37°C.

Animals - Use overtly healthy, mature rabbits. For a normal strain that is commonly used (New Zealand Whites) each should weigh not less than 1500 g. House the animals individually in an area of uniform temperature [± 3°C. (± 5° F.)] and free from disturbances likely to excite them. Before using an animal for the first time in a pyrogen test, condition it by a sham test that includes all of the steps as directed under

MATERIALS FOR TESTING Con't.

"Test Procedure" except the injection of the test dose. Do not use animals for pyrogen tests more frequently than once every 48 hours, nor prior to 2 weeks following their having been given a test sample that was adjudged pyrogenic. For human blood products the rabbits are selected from the special "Blood Colony" for one test only at the present time.

Temperature Recording - Use an accurate clinical thermometer for which the time necessary to reach the maximum reading is known, or any other temperature-recording device of equal sensitivity. Insert the thermometer or probe into the rectum of the test animal to a depth of not less than 7.5 cm. and after a period of time not less than that previously determined as sufficient, record the animal's body temperature.

Reagents - Pyrogen free normal saline - Supplied by Production Specification No. 3220
Sterile Water for Injection - U.S.P.

TEST PROCEDURE

Perform the test under environmental conditions similar to those under which the animals are housed. During the test, withhold all food from the animals being used. Access to water may be allowed. If rectal temperature measuring probes are to remain inserted throughout the testing period, restrain the rabbits with light-fitting neck stocks that allow the rabbits to assume a natural resting posture.

Not more than 40 minutes prior to the injection of the test dose, determine the "control temperature" of each animal; this is the base for the determination of any temperature increase resulting from the injection of a test solution. In any one test use only those animals the control temperatures of which do not vary by more than 1°C. from each other, and do not use any animal with a temperature exceeding 39.8°C.

Unless otherwise specified, inject into an ear vein of each of three rabbits 10 ml. of the product per kg. of body weight, completing the injection within 10 minutes after start of administration. Record the temperature at 1, 2, and 3 hours subsequent to the injection.

AP000642

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Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD No. 208
ANALYTICAL METHOD	PYROGEN TEST

INTERPRETATIONS

Record observed temperature decreases as zero. If no rabbit shows an individual rise in temperature of 0.6°C. or more above its respective control temperature, and if the sum of the three individual maximum temperature rises does not exceed 1.4°C., the product meets the requirements for the absence of pyrogens. If any rabbit shows an individual temperature rise of 0.6°C. or more, or if the sum of the three individual maximum temperature rises exceeds 1.4°C repeat the test using five other rabbits. If not more than three of the eight rabbits show individual rises in temperature of 0.6°C. or more, and if the sum of the eight individual maximum temperature rises does not exceed 3.7°C. the material under examination meets the requirements for the absence of pyrogens.

REFERENCES

U.S.P.; Code of Federal Regulations, 21, Section 610.13, 1976

AP000643

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD NO. 208 Supplement #38
ANALYTICAL METHOD	PYROGEN TESTING OF ANTIHEMOPHILIC FACTOR (HUMAN), GENERATION II-B, LYOPHILIZED

DATE	SUPERSEDES:	PREPARED BY:
9/18/80	4/11/80	A. K. Roop

PREPARATION

Reconstitute a vial of product with 30 ml of sterile water for injection using the solution procedure as stated in Method No. 1343. Assuming 1000 U/Vial,* dilute the reconstituted sample 1:3.3 with sterile 0.9% saline to produce a final solution containing 10 U/ml.

DOSE

Inject intravenously 4 ml/Kg body weight or 40 U/Kg body weight.

*If upon completion of the potency assay it is discovered:

1. That the vial potency is 850 U/V or less, the test is invalid and will be performed on the actual potency.

2. That a lot is pyrogenic and the assayed potency is in excess of the 1000 U/Vial assumed potency, the pyrogen test will be invalidated and a new test will be repeated with the test dose adjusted to 40 U/Kg based on the assayed potency.

REFERENCE

Armour Method No. 208.

7/17/80
mml

AP000644

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD NO. 208 Supplement #41
ANALYTICAL METHOD	ANTIHEMOPHILIC FACTOR (HUMAN), GENERATION II-B, LYOPHILIZED (APPROXIMATELY 500 U/VIAL)

DATE	SUPERSEDES:	PREPARED BY:
3/19/80	New	A. K. Roop

PREPARATION

Reconstitute a vial of product with 20 ml of sterile water for injection using the solution procedure as stated in Method No. 1343. After the sample is completely in solution, add 30 ml of sterile 0.9% saline using a sterile syringe to produce a final volume of 50 ml and a concentration of 10 U/ml.

DOSE

Assuming a potency of 500 units/vial, inject intravenously 4 cc/Kg. body weight or 40 units/Kg. body weight.

REFERENCE

Armour Method No. 208.

3/5/80
tw

AP000645

Armour Pharmaceutical Company
QUALITY STANDARDS
Kankakee, Illinois

ANALYTICAL METHOD

APC METHOD NO. 208
Supplement #42

ANTIHEMOPHILIC FACTOR (HUMAN),
GENERATION II-B, LYOPHILIZED
(APPROXIMATELY 250 U/VIAL)

DATE:	SUPERSEDES:	PREPARED BY:
3/19/80	New	A. K. Roop

PREPARATION

Reconstitute a vial of product with 10 ml of sterile water for injection using the solution procedure as stated in Method No. 1343. After the sample is completely in solution, add 15 ml of sterile 0.9% saline using a sterile syringe to produce a final volume of 25 ml and a concentration of 10 U/ml.

DOSE

Assuming a potency of 250 units/vial, inject intravenously 4 cc/Kg. body weight or 40 units/Kg. body weight.

REFERENCE

Armour Method No. 208.

3/5/80
tw

AP000646

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD NO. 303 STERILITY TESTING - FINAL PRODUCT
ANALYTICAL METHOD	

DATE:	SUPERSCDES:	PREPARED BY:
10/9/80	New	L. Cotter

TEST SUMMARY

The samples taken from final containers are implanted in Fluid Thioglycollate and Soybean-Casein Digest*, and incubated for not less than 14 days. After the specified time, if no growth is visible the product is found to meed the requirements for sterility.

COMMENTS

a. Safety Precautions: Follow general laboratory safety rules.

b. General Precautions: All sterility testing must be conducted in an aseptic area by trained personnel.

Rigid aseptic techniques must be employed at all times. Testing should NOT be conducted in areas under aerosol treatment. Environmental control tests, such as exposure plates, must be performed daily in the aseptic sterility testing area.

Freshly prepared media, if not used within 2 days, must be stored in the dark, preferably at 2-25°. Finished media may be stored in unsealed containers for more than 10 days, provided they are tested weekly for growth promotion. If stored in sealed containers, the media may be used for one year, provided they are tested for growth promotion every 3 months.

In order to avoid false negative results, the bacteriostatic and fungistatic activity for each product must be established. If the product is bacteriostatic or fungistatic, a suitable sterile inactivating agent must be used or in the absence of such an agent, the established product inoculum media ratios must be adhered to (Method 309).

- continued on next page -

*BBL designation is trypticase soy broth

AP000647

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD No. 303
ANALYTICAL METHOD	STERILITY TESTING - FINAL PRODUCT

COMMENTS (Con't.)

Growth Promoting Test of Media

Each lot of medium is tested for sterility and its growth-promoting qualities. Inoculate two sets of the test medium with not more than 100 spores of Bacillus subtilis (ATCC No. 6633). Likewise, inoculate two sets of test medium with the same number of organisms of Candida albicans (ATCC No. 10231). For Fluid Thioglycollate Medium only, test also two additional sets of medium with no more than 100 organisms of Bacteriodes vulgatus (ATCC No. 8482). Additional organisms may be used. Incubate all inoculated sets of Soybean-Casein Digest medium at 20-25°C and those of Fluid Thioglycollate Medium at 30-32°C. The test media are satisfactory if evidence of substantial growth appears within 7 days. These tests may be conducted simultaneously with the use of the test media provided; the sterility test is considered unsatisfactory if the test medium shows poor or no growth response (Method 410).

Confirm the sterility of every lot of medium used by incubating samples at the temperature and time specified in the method.

MATERIALS FOR TESTING

1. Bacillus subtilis (ATCC No. 6633)
2. Candida albicans (ATCC No. 10231)
3. Bacteriodes vulgatus (ATCC No. 8482)
4. Soybean-Casein Digest made according to USP or purchased commercially
5. Fluid Thioglycollate Medium made according to USP or purchased commercially
6. Media tubes

TEST PROCEDURE

Product Sampling

For products which are sterilized with steam under pressure in the final sealed containers, select 20 or more units from each sterilizer load. These samples must be representative of all layers of the load. For all other products, select a total of 20 or more units representative of each batch, taken at regular intervals throughout each filling operation.

When testing a lyophilized product, reconstitution should be done according to the directions supplied with the product.

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD No. 303
ANALYTICAL METHOD	STERILITY TESTING - FINAL PRODUCT

TEST PROCEDURE (Con't.)

Testing Techniques

1. Liquids and Suspension

   a. Sterilize the exterior surfaces of vials and ampules with a suitable bactericidal agent.

   b. Open ampules by breaking off neck with sterile gloved fingers.

   c. Remove liquids or suspensions for culturing with a sterile pipette or with a sterile syringe fitted with a sterile hypodermic needle.

   d. Plant portions of the material from each container being tested into Fluid Thioglycollate Medium. In addition, also plant portions from each container into Soybean-Casein Digest Medium (See chart under Inoculum Size). If the volume is not sufficient for seeding both the Fluid Thioglycollate and Soybean-Casein Medium, use duplicate containers.

   If the product contains mercurial preservative, replace the Soybean-Casein Digest Medium with another tube of Fluid Thioglycollate Medium and incubate 14 days at 20-25°C.

2. Crystalline and Powdered Solids

   If the product is soluble or dispersible, the suitable amount of sterile diluent is added aseptically to the final container. After mixing, withdraw a quantity of the product corresponding to 300 mg from each container being tested, or the entire contents if less than 300 mg, and transfer to 80 ml Fluid Thioglycollate Medium and 80 ml Soybean-Casein Digest Medium, respectively, and mix.

3. Oils and Ointments

   Select 20 containers, assign them to 2 groups of 10 containers, and treat each group as follows. Aseptically transfer 100 mg from each of the 10 containers to a flask containing 100 ml of a sterile aqueous vehicle capable of dispersing the test material homogeneously throughout the fluid mixture.

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AP000649

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Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD No. 303
ANALYTICAL METHOD	STERILITY TESTING - FINAL PRODUCT

TEST PROCEDURE (Con't.)

Testing Techniques (Con't.)

3. Oils and Ointments (Con't.)

NOTE: The choice of dispersing agent incorporated in the aqueous vehicle may differ according to the nature of the ointment or oil. Before use, test the dispersing agent to ascertain that in the concentration used it has no significant antimicrobial effects during the time interval for all transfers. Mix 10 ml of the fluid mixture so obtained with 80 ml of medium, and proceed as directed under Liquids.

Inoculum Size

Vary the minimum volume of medium used according to the content of the final container as follows:

Biologicals

CONTAINER CONTENT	MINIMUM VOL. OF PRODUCT	MINIMUM VOL. OF MEDIUM IF PRESERVATIVE	MINIMUM VOL. OF MEDIUM IF NO PRESERVATIVE
10 ml or less	1 ml or total content if less than 1 ml	80 ml	80 ml
From 10 to 50 ml	5 ml	80-120 ml	80 ml
More than 50 ml	10 ml	250 ml	80-250 ml

All Other Final Products

CONTAINER CONTENT	MINIMUM VOL. OF PRODUCT	MINIMUM VOL. OF MEDIUM IF PRESERVATIVE	MINIMUM VOL. OF MEDIUM IF NO PRESERVATIVE
Less than 10 ml	1 ml or total contents if less than 1 ml	80 ml	80 ml
10 to 49 ml	5 ml	80-250 ml	80 ml
50 ml or more	10 ml	250 ml	80-250 ml

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD No. 303
ANALYTICAL METHOD	STERILITY TESTING - FINAL PRODUCT

TEST PROCEDURE (Con't.)

Incubation

Incubate the Fluid Thioglycollate at 30-32°C and the Soybean-Casein Digest Medium at 20-25°C for not less than 14 days.

When the material to be tested renders the medium turbid, so that the presence or absence of growth cannot be determined readily by visual examination, transfer between the third and seventh days, suitable portions of this turbid medium to additional tubes of medium.

Incubate both the original and sub-culture tubes for not less than 7 additional days after the transfer and for a total of not more than 14 days.

Examine tubes daily and at the end of the incubation period for the presence of growth. All tubes showing growth are verified by microscopic examination of stained smears (Armour Method 310).

If no growth is found, the material under examination meets the requirments for STERILITY. If evidence of microbial growth is found the material tested fails to meet the requirements of the test for STERILITY, unless it can be demonstrated by retests or by other means that the test was invalid for causes unrelated to the article.

In view of the possibility that microbial growth observed in the test was due to inadequate aseptic sampling and testing technique rather than to intrinsic contamination of the article, the following retests are permitted.

Complete the attached form and file with Quality Assurance - Product Control (S.O.P. C-28).

First Retest

The number of specimens selected, the volumes to be tested, and the media are the same as those indicated for the original STERILITY TEST. If no evidence of microbial growth is found, the material tested meets the requirements of the test for STERILITY. If microbial growth appears in this First Retest, isolate and characterize the microbial contaminant(s) of the

- continued on next page -

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD No. 303
ANALYTICAL METHOD	STERILITY TESTING - FINAL PRODUCT

TEST PROCEDURE (Con't.)

Incubation (Con't.)

First Retest (Con't.)

First Retest and compare to the contaminant(s) of the original STERILITY TEST. If the contaminant(s) cannot be differentiated readily, the material tested fails to meet the requirements of the test for STERILITY. If the contaminant(s) can be differentiated readily, a Second Retest may be performed.

Complete the attached form and file with Quality Assurance - Product Control (S.O.P. C-28).

Second Retest

The number of specimens selected is double the number tested in the original STERILITY TEST and in the First Retest. The volumes tested from each specimen and the media are the same as those indicated for the original STERILITY TEST and the First Retest.

If no evidence of microbial growth is found, the material tested meets the requirments of the test for STERILITY. If growth appears in this Second Retest, the material tested fails to meet the requirements of the test for STERILITY.

NOTE: For interpretation of allowable results for human blood products, see 21 CFR 610.12 (a2) (b).

REFERENCES

United States Pharmacopeia, XX

Code of Federal Regulations, Title 21, Part 610, Section 610.12.

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Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD NO. 351 MAMMALIAN PROTEIN SPECIES IDENTIFICATION (AGAR DIFFUSION)
ANALYTICAL METHOD	

DATE:	SUPERSEDES:	PREPARED BY:
10/12/77	New	R. H. Brown

TEST SUMMARY:

The agar diffusion test is used to demonstrate the presence or absence of human, bovine, ovine or porcine proteinaceous material in human blood fractions. In the agar diffusion method, an antiserum containing specific antibodies is diffused with a material which is suspected of containing the corresponding antigen.

COMMENTS:

A. Safety Precautions:

1. Personal hygiene cannot be overemphasized. Special awareness of this practice should be noted when handling human blood derivatives since there exists an inherent risk of hepatitis infection.

2. Practice general laboratory safety regulations.

B. General Precautions:

1. If two different antiserums are placed in opposing wells, the antiserums will usually react with one another due to the presence of antigens used to absorb each antiserum.

2. Room temperature is the most suitable for this test procedure. Reactions appear too quickly and are fainter at 37°C. Refrigerator temperatures (2 to 8°C.) cause a slower reaction rate. In fact, in some cases, the expected reaction of a known positive never appears.

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD No. 351 MAMMALIAN PROTEIN SPECIES IDENTIFICATION (AGAR DIFFUSION)
ANALYTICAL METHOD	

MATERIALS FOR TESTING

A. Antiserums to be Used

1. Rabbit Anti-Ovine Serum
2. Rabbit Anti-Bovine Serum
3. Rabbit Anti-Porcine Serum
4. Rabbit Anti-Human Serum

All antiserums should be checked out for cross-reactions with other animal proteins. If any cross-reactions are noted, an antiserum may be specifically absorbed with appropriate protein (antigen). Antiserums are supplied through commercial distributors.

B. Controls

1. Positive Ovine Control - 25% human albumin prepared to contain approximately 70 ppm ovine albumin.
2. Positive Bovine Control - 25% human albumin prepared to contain approximately 19 ppm bovine albumin.
3. Positive Porcine Control - 25% human albumin prepared to contain approximately 100 ppm porcine albumin.
4. Negative Control - 25% human albumin previously tested and known to be negative against ovine, bovine and porcine antisera.

C. Equipment Necessary

1. Hyland Immuno-Plate^{R} Immunodiffusion Plates (Ouchterlony), Pattern D
2. Capillary pipettes
3. Saline Solution - 0.9% sodium chloride. Dissolve 9 g. sodium chloride in 1000 ml distilled water.
4. Moist Chamber - Chamber with moist piece of sponge or filter paper.

TEST PROCEDURE

A. Preparation of Samples for Testing

1. Albumins are ordinarily tested as a 25% solution, and, if necessary, are diluted to this percentage with saline solution. (1.25 g. powder are dissolved in 5 ml. saline solution).
2. Gamma Globulins are ordinarily tested as a 16% solution. (0.8 g. powder are dissolved in 5 ml. saline solution).
3. Other blood products to be prepared as described on the package.

Armour Pharmaceutical Company
QUALITY STANDARDS
Kankakee, Illinois

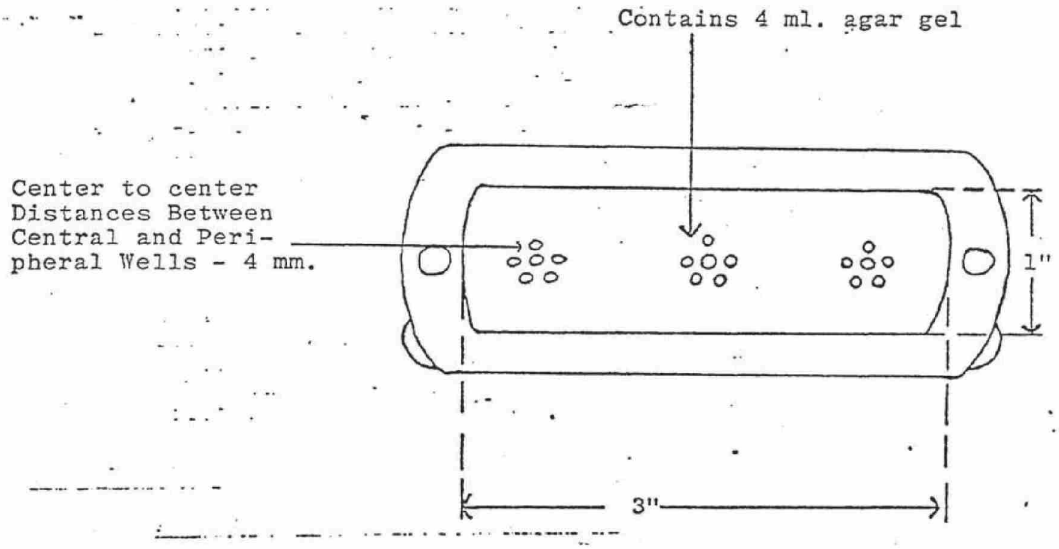
APC METHOD No. 351

MAMMALIAN PROTEIN SPECIES IDENTIFICATION (AGAR DIFFUSION)

ANALYTICAL METHOD

B. Double Diffusion Agar Gel Plates

Use Hyland Immuno-Plate^{R} Immunodiffusion plates (Ouchterlony), Pattern D. The agar gel consists of Difco special 2% Noble agar, 7.5% glycine, 1% sodium chloride, 0.1% sodium azide with a pH of 7.0 to 7.2. The following diagram shows dimensions:



This pattern plate was chosen because of the speed with which the reactions occur. A final reading can usually be made within 6 hours. However, plates are held 24 hours before a test is recorded as negative. Caution must be exercised because of the speed of the reactions. Plates should be observed every half hour for the first 6 hours, and occasionally thereafter until 24 hours has elapsed. With familiar products a check at every half hour is unnecessary, however for new products a check is essential.

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Armour Pharmaceutical Company
QUALITY STANDARDS
Kankakee, Illinois

APC METHOD No. 351

MAMMALIAN PROTEIN SPECIES IDENTIFICATION (AGAR DIFFUSION)

ANALYTICAL METHOD

C. Set-Up and Assay

1. Using capillary pipettes, fill wells of agar plates in predetermined manner with samples to be tested and also controls.

2. Antigen is usually placed in outer wells; antiserum in center well. This conserves antiserum.

3. Place filled plates in moist chamber and incubate at room temperature.

4. Examine plates as necessary for 6 hours for precipitin reactions, i.e., an opaque zone or "line" between the antiserum well and each antigen well. (Indirect lighting may be used for this examination.)

5. Record positive results if they appear within 6 hours.

6. Examine the plate occasionally (for the next 18 hours).

RESULTS:

If at the end of 24 hours no reaction has appeared the test is negative.

If excess antigen or antibody exists, the reaction will appear very quickly (½ to 1½ hours), but may completely disappear by 6 hours or more. Positive reactions should be recorded as they appear and are considered a positive test even if the reaction disappears.

For the test to be valid, positive controls must be functioning properly; that is a positive reaction with the specific antiserum and no cross-reactions with other antiserums except Rabbit Anti-Human Albumin.

REFERENCES:

Armour Method No. 351.

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Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD NO. 386
ANALYTICAL METHOD	DETERMINATION OF ISOAGGLUTININ TITERS IN ANTIHEMOPHILIC FACTOR (HUMAN)

DATE	SUPERSEDES:	PREPARED BY:
3/14/80	New	R. Kleszynski

TEST SUMMARY

Determine the antiglobulin titer for AHF by incubating washed type A and type B red blood cells with the test material and measuring the degree of cell agglutination which occurs.

COMMENTS

Safety Precautions: Personal hygiene cannot be overemphasized. Special awareness of this practice should be noted when handling blood derivatives, since there exists an inherent risk of hepatitis infection.

MATERIALS FOR TESTING

1. 12 x 75 mm test tubes and racks
2. Physiological saline (9 gms/L.)
3. Adams Sero-fuge
4. Agglutination viewer with light and mirror
5. Coombs serum
6. Eppendorf pipette 0.1 ml and disposable tips
7. 37°C. water bath
8. Fresh anticoagulated whole blood type A and type B
9. Control serum - O serum or plasma with a known A & B titer
10. AHF samples to be tested - Reconstituted According to Label

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD No. 386
ANALYTICAL METHOD	DETERMINATION OF ISOAGGLUTININ TITERS IN ANTIHEMOPHILIC FACTOR (HUMAN)

TEST PROCEDURE

A. Preparation of Cell Suspensions

1. Type A and Type B cell suspension are prepared by pooling fresh blood from 3 different A & B donors.

2. The Red Blood Cells (RBC's) are washed with saline, a minimum of three times in an Adams Sero-fuge, spinning one minute at each wash. The supernatant is discarded after each wash.

3. Prepare a 2% cell suspension in saline by pipetting 0.2 ml. of each of the washed cell preparations into tubes containing 9.8 mls. of saline. Mix well.

B. Anti-A and Anti-B 37°C. Saline Titers

1. Label 4 rows of 12 x 75 mm test tubes according to the serum dilution--usually 1:1 through 1:512.

2. Label the first tube in row 1 "sample-A"; the first tube in row 2 "control-A"; the first tube in row 3 "sample-B"; and the first tube in row 4 "control-B".

3. Use an automatic pipette to deliver 0.1 ml. of saline into the bottom of all tubes except the first tube in each row.

4. Pipette 0.1 ml. of sample being tested into tubes 1 and 2 of the first and third sample rows. Pipette 0.1 ml. of the serum control into tubes 1 and 2 of the second and fourth control rows.

5. With a clean pipette, mix the contents of tube 2 (1:2) in row one several times. Transfer 0.1 ml. to tube labeled 4 (1:4 dilution) in the same row.

6. Continue same procedure through dilutions 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, and 1:512. A separate pipette must be used for each dilution if "carryover" of antibody from one tube to the next is to be avoided.

7. Repeat procedures 5 and 6 on the next three rows.

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD No. 386
ANALYTICAL METHOD	DETERMINATION OF ISOAGGLUTININ TITERS IN ANTIHEMOPHILIC FACTOR (HUMAN)

TEST PROCEDURE

B. 8. Pipette 0.1 ml. of the saline suspension of A red blood cells into all tubes in rows 1 and 2; pipette 0.1 ml. of the saline suspension of B red blood cells into all tubes in rows 3 and 4. Shake tubes well.

9. Incubate all tubes for 15 minutes at 37°C.

10. Centrifuge all tubes in Adams Sero-fuge for 45 seconds.

11. The formed cell button is gently dislodged, observed macroscopically and graded as follows:

4+ Cell button remains in one clump
3+ Cell button dislodges into several clumps
2+ Cell button dislodges into many small clumps of nearly equal size
1+ Cell button dislodges into finely granular, but definite, small clumps
0 Cell button dislodges with an absence of discernible clumps

The endpoint is expressed at the dilution of which 1+ agglutination is seen and reported as the Anti-A and Anti-B 37°C. saline titer.

C. Coombs Antiglobulin Titer

1. Wash all negative and 1+ tubes 3 times with saline, centrifuging 45 seconds after each wash.

2. After the third washing, perform an antiglobulin (Coombs) test by adding 2 drops commercial Coombs serum to each tube and centrifuge 45 seconds.

3. Again observe macroscopically for agglutination using the same criteria as in Step 11, Section B, of TEST PROCEDURE.

4. Titers on the control serum should be in the expected range to assure assay results on the product being tested are correct.

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD No. 386
ANALYTICAL METHOD	DETERMINATION OF ISOAGGLUTININ TITERS IN ANTIHEMOPHILIC FACTOR (HUMAN)

REFERENCES

Technical Methods and Procedures of the AABB, Fifth Edition, 1970, Reprinted, 1973.

Bray's Clinical Methods and Procedures, Bauer, Ackermann, Toro. Seventh Edition, Copyright 1968, C.V. Mosley Company.

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ARMOUR PHARMACEUTICAL COMPANY
QUALITY CONTROL DEPARTMENT
ANALYTICAL METHODS

METHOD NUMBER 963

GENERAL SAFETY TEST

Product To Be Tested:

The general safety test shall be conducted upon a representative sample of the product in the final container from every final filling of each lot of the product. If any product is processed further after filling, such as by freeze-drying, sterilization, or heat treatment, the test shall be conducted upon a sample from each filling of each drying chamber run, sterilization chamber, or heat treatment bath.

Test Animals:

Only overtly healthy guinea pigs weighing less than 400 grams each and mice weighing less than 22 grams each shall be used. The animals shall not have been used previously for any test purpose.

Procedure:

The duration of the general safety test shall be 7 days for both species, except that a longer period may be established for specific products in accordance with the following paragraph entitled Test Variations. After a specific duration of the test period for a specific product has been established, it cannot be varied subsequently, except in accordance with the paragraph entitled Test Variations. Each test animal shall be weighed and the individual weights recorded immediately prior to injection and on the last day of the test. Each animal shall be observed every working day. Any animal response including any which is not specific for or expected from the product and which may indicate a difference in its quality shall be recorded on the day such response is observed. The test product shall be administered as follows:

1. Liquid product or freeze-dried product which has been reconstituted as directed on the label. Inject intraperitoneally 0.5 milliliter of the liquid product or the reconstituted product into each of at least two mice; and 5.0 milliliters of the liquid product or the reconstituted product into each of at least two guinea pigs.

2. Freeze-dried product for which the volume of reconstitution is not indicated on the label. The route of administration, test dose, and diluent shall be as approved by the Director, Bureau of Biologics, in accordance with the paragraph entitled Test Variations. Administer the test product as approved on at least two mice and at least two guinea pigs.

3. Non-liquid products other than freeze-dried product. The route of administration, test dose, and diluent shall be as approved by the Directo Bureau of Biologics, in accordance with the paragraph entitled Test Variations. Dissolve or grind and suspend the product in the approved diluent. Administer the test product as approved on at least two mice and at least two guinea pigs.

Test Requirements:

A safety test is satisfactory if all animals meet all of the following requirements:

1. They survive the test period.
2. They do not exhibit any response which is not specific for or expected from the product and which may indicate a difference in its quality.
3. They weigh no less at the end of the test period than at the time of injection.

Repeat Tests:

1. First repeat test. If a filling fails to meet the requirements of the Test Requirements in the initial test, a repeat test may be conducted on the species which failed the initial test, as prescribed in Procedure The filling is satisfactory only if each retest animal meets the requirements prescribed in Test Requirements.

2. Second repeat test. If a filling fails to meet the requirements of the first repeat test, a second repeat test may be conducted on the species which failed the test; provided that 50 percent of the total number of animals in that species has survived the initial and first repeat tests. The second repeat test shall be conducted as prescribed in the Procedure except that the number of animals shall be twice that used in the first repeat test. The filling is satisfactory only if each second repeat tes animal meets the requirements prescribed in paragraph Test Requirements.

Test Variations:

Variations in the general safety test, such as test dose, route of administration, or duration of the test period may be offered as an amendment to the product license and must receive written approval by the Director, Bureau of Biologics, Food and Drug Administration. Approval will be given only if the license amendment provides substantial evidence demonstrating that the proposed test variation will assure sensitivity equal to or greater than the te prescribed in this method.

Reference: Code of Federal Regulations, Title 21, Paragraph 610.11

QUALITY CONTROL DEPARTMENT
ANALYTICAL METHODS

METHOD NUMBER 993

BIURET ASSAY FOR TOTAL PROTEIN CONTENT OF CRYOPRECIPITATED ANTIHEMOPHILIC GLOBULIN (AHF)

I. Reagents:

1. Standard Protein Solution (3 mg./ml.)
   Dissolve 300 mg. of crystallized human albumin in 75 ml. of distilled water and dilute to 100 ml. with distilled water. The solution should be stored at 2 - 8°C. and is stable for 2 months.

2. 3 Normal Sodium Hydroxide (NaOH)
   Dissolve 120 gms. of sodium hydroxide pellets in 750 ml. of distilled water and dilute to 1 liter with distilled water.

3. 6 Normal Sodium Hydroxide (NaOH)
   Dissolve 240 gms. of sodium hydroxide pellets in 750 ml. of distilled water and dilute to 1 liter with distilled water.

4. Biuret Reagent
   a. Dissolve 17.3 gms. of copper sulfate (anhydrous) in 75 ml. of distilled water and dilute to 100 ml. with distilled water.

   b. Dissolve 173 gms. of sodium citrate dihydrate and 100 gms. of sodium carbonate (anhydrous) in 700 ml. of distilled water. Warm the solution to facilitate solution of the reagents.

   c. Cool the two solutions and pour the copper sulfate into the sodium citrate - sodium carbonate mixture.

   d. Stir and dilute to 1 liter with distilled water.

   e. The Biuret reagent is stable indefinitely at room temperature.

II. Preparation of Protein Standard:

1. Label a series of 5 test tubes in triplicate as follows:
   a. 1.5 mg./ml.
   b. 3 mg./ml.
   c. 4.5 mg./ml.
   d. 6 mg./ml.
   e. 7.5 mg./ml.

2. To A, add 0.5 ml. of the standard protein solution.
   To B, add 1.0 ml. of the standard protein solution.

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II. Preparation of Protein Standard: - continued

To C, add 1.5 ml. of the standard protein solution.
To D, add 2.0 ml. of the standard protein solution.
To E, add 2.5 ml. of the standard protein solution.

3. To all test tubes add equal amounts of 6 Normal sodium hydroxide.

4. To A, B, C, and D, add the required amounts of 3 Normal sodium hydroxide to bring the total volume to 5.0 ml.

Note: E will already be at the required volume of 5.0 ml.

5. To all 5 test tubes, add 1.0 ml. of the Biuret reagent and mix well.

6. Prepare a blank by mixing 5 ml. of the 3 Normal sodium hydroxide and 1.0 ml. of the Biuret reagent and mix well.

7. Use the blank to standardize the spectrometer and determine the optical density of each standard solution at 545 nm.

8. Plot the results on linear graph paper, optical density vs. concentration.

III. Total Protein of Test Sample:

1. The amount of sample to be used is estimated so that its value will fall on the standard curve. This is usually done by trial runs. It has been found that the following sample size can be used to obtain readings on the standard curve.

a. AHF cryoprecipitated sample - If the contents of a vial are reconstituted to 25 ml., the estimated concentration of protei is 15-16 mg/vl. Dilute 2.0 ml of this solution to 10 ml. Use 1.0 ml for the assay.

2. To the amount of sample used, add equal amounts of 6 Normal sodium hydroxide. Prepare the test sample in triplicate.

3. Dilute to 5 ml. with 3 Normal Sodium Hydroxide.

4. Add 1.0 ml. of Biuret reagent and mix well.

5. Prepare blank in same manner as for the standard curve.

6. Read at the same wave length as for the standard curve.

7. Calculate the amount of protein in the test sample from the standar curve. Correct for sample dilution and express the total protein content in mg./AHF unit.

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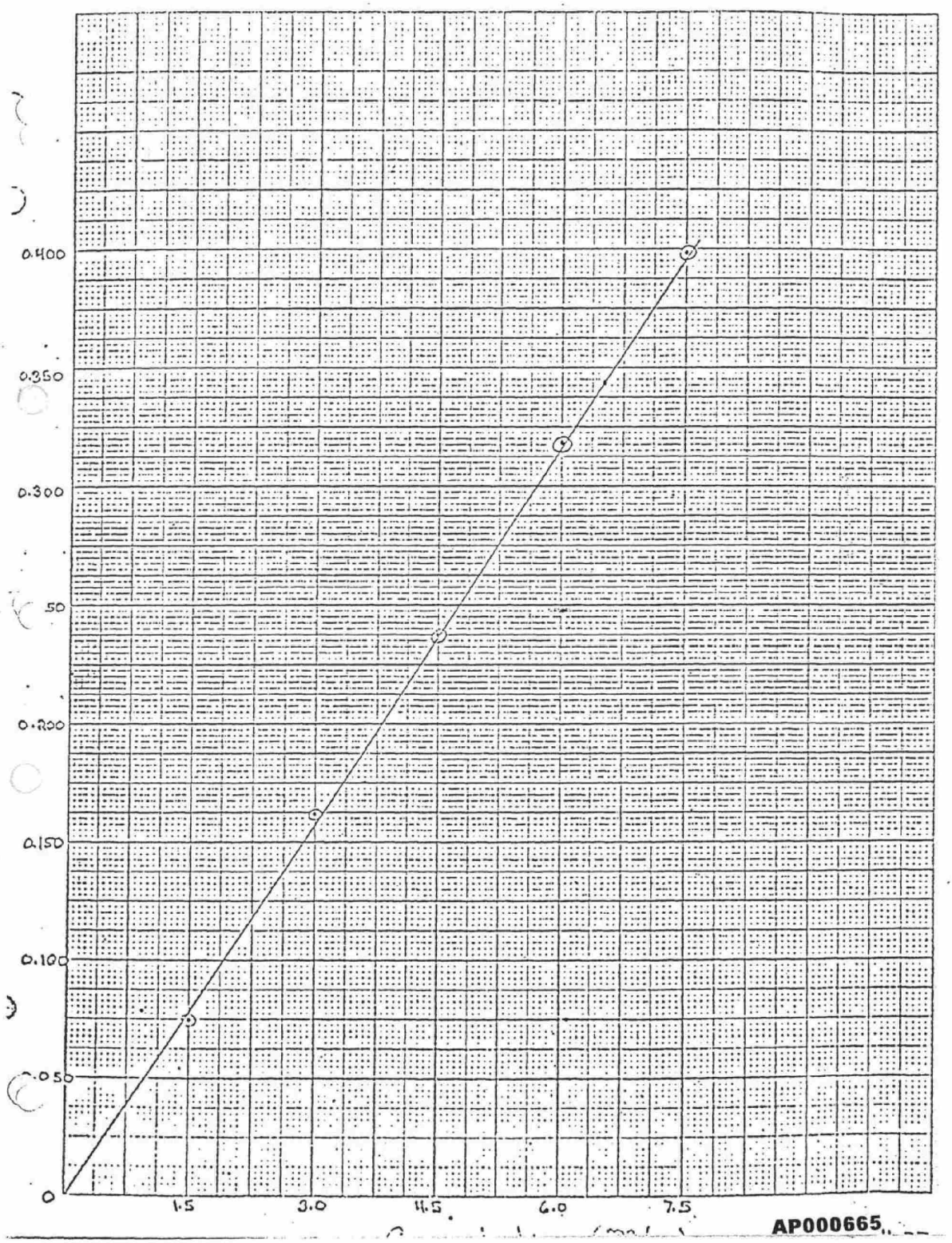

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Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD NO. 995
ANALYTICAL METHOD	ATOMIC ABSORPTION-ANALYSIS ALUMINUM IN ANTIHEMOPHILIC FACTOR

DATE:	SUPERSEDES:	PREPARED BY:
1/28/81	New	Art Roop

TEST SUMMARY

A solution of the sample is aspirated into the nitrous oxide-acetylene flame of an atomic absorption spectrophotometer where the aluminum in the sample is ionized. Energy originating from an aluminum filament hollow cathode lamp and directed through the flame of the instrument is absorbed by the aluminum ions present in the flame. The absorption of energy is proportional to the concentration of aluminum ions present in the flame. The aluminum present in the sample is determined by comparing the absorption of energy due to the sample to that obtained from a series of solutions containing known concentrations of aluminum.

COMMENTS

Safety Precautions - Lack of understanding of proper ignition and shut down procedure of the nitrous oxide-acetylene flame can result in personal injury and damage to the instrument. Before operation of the instrument, read the manufacturers operating instructions and be instructed in the proper procedure for flame ignition and shut down.

General Precautions - None.

MATERIALS FOR TESTING

1. Perkin-Elmer, Model 303, Atomic-Absorption Spectrophotometer equipped with an aluminum hollow cathode lamp and a nitrous oxide burner head. An equivalent unit may be used.

2. Perkin-Elmer Recorder Readout or equivalent.

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD No. 995
ANALYTICAL METHOD	ATOMIC ABSORPTION-ANALYSIS ALUMINUM IN ANTIHEMOPHILIC FACTOR

MATERIALS FOR TESTING(Con't.)

3. Omni Scribe Recorder (Houston Instruments) or equivalent.

4. Volumetric glassware as needed.

5. Aluminum Standard Solution, 1000 ppm, purchased from Harleco, Item No. 7689 or equivalent.

6. Sodium Chloride Solution, 1000 ppm. Dissolve 1 g of Analytical Reagent Grade Sodium Chloride in 1 liter of water.

7. Nitrous oxide compressed gas.

8. Acetylene compressed gas.

9. Necessary control valves and fittings for connecting the compressed gases to the instrument.

REAGENTS

Preparation of Standard Aluminum Solutions:

Transfer a 2 ml aliquot of the Aluminum Standard Solution, 1000 ppm, to a 200 ml volumetric flask, dilute to volume with Sodium Chloride Solution, 1000 ppm, and mix well. Transfer a 10 and 20 ml aliquot to two 100 ml volumetric flasks, respectively, and dilute each to volume with Sodium Chloride Solution, 1000 ppm, to produce two final solutions containing 1 and 2 ppm aluminum. Mix well.

Preparation of Sample Solution:

Refer to the following table of Specification Nos. to determine the volume of Sodium Chloride Solution, 1000 ppm required to reconstitute the sample being tested.

SPECIFICATION NO.	VOLUME FOR RECONSTITUTION
3072	25 ml
3086	30
3453	25
3502	50
3807	30
3913	10
3914	20

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Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD No. 995 ATOMIC ABSORPTION-ANALYSIS ALUMINUM IN ANTIHEMOPHILIC FACTOR
ANALYTICAL METHOD	

FLAME IGNITION PROCEDURES

NOTE: The following Flame Ignition Procedures are presented by Perkin-Elmer. The analyst is advised to read the instruction manual and the General Information section of the Perkin-Elmer Analytical Methods book before using the instrument.

With the exception of the nitrous oxide-acetylene flame, all flames may be ignited directly as described in the instruction manual for the appropriate instrument.

Always turn fuel on last and off first.

Experience has shown that with the nitrous oxide-acetylene flame, flashback is most likely to occur when the flame is either ignited or turned off. These flashbacks can generally be avoided if the flame is turned on or off with air as the oxidant. This procedure requires a means of rapidly switching from air to nitrous oxide. Most instrument gas control systems now have this capability. For those which do not, a T-junction valve is available as an acessory (Perkin-Elmer part number 303-0225). The ignition sequence given below for the nitrous oxide-acetylene flame is usable on all Perkin-Elmer atomic absorption spectrophotometers except those equipped with gas control boxes providing automatic switchover to nitrous oxide (gas control boxes 040-0301, 057-0134, 057-0345, and 057-0262). With gas control boxes providing automatic switchover, it is merely necessary to install the nitrous oxide burner head. All secondary acetylene adjustments take place automatically.

1. Install a nitrous oxide burner head.

2. Turn on the acetylene flow (without igniting the flame) and adjust the flow rate to the appropriate value specified for the nitrous oxide-acetylene flame. Turn the acetylene flow off.

3. With both air and nitrous oxide supplies turned on, set the switching valve to nitrous oxide and adjust the flow rate to the value specified.

4. Turn the switching valve to the air position.

5. Turn the acetylene on and ignite the flame (air-acetylene). Allow the burner head to warm up for several minutes.

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Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD No. 995
ANALYTICAL METHOD	ATOMIC ABSORPTION-ANALYSIS ALUMINUM IN ANTIHEMOPHILIC FACTOR

FLAME IGNITION PROCEDURES continued

6. Increase the acetylene flow to the value specified for nitrous oxide-acetylene operation in the appropriate table.

7. With a rapid motion, turn the switching valve from air to nitrous oxide.

The nitrous oxide flame is now operating normally. Fuel and oxidant flow can be adjusted as described under Gas Flow Optimization.

When extinguishing the flame, the reverse procedure is followed. The oxidant switching valve is switched rapidly from the nitrous oxide to the air position, after which the acetylene flow is reduced, then turned off.

TEST PROCEDURE

Using a Perkin Elmer Model 303 Atomic Absorption Spectrophotometer, or equivalent, equipped with an aluminum lamp and a nitrous oxide burner head, determine the absorption values for the sample and standard solutions versus a blank solution consisting of a solution of 1000 ppm Sodium Chloride. Suggested instrument settings for the instruments being used are:

Perkin-Elmer, Model 303 Atomic Absorption Spectrophotometer
Wavelength - 309.9 nanometers
Range - UV
Slit - 4
Source - As recorded on the lamp
Scale - 1
Oxidizer - Nitrous Oxide - Air - 6.0
Fuel Flow - Initially white ball at 8, light flame, increase flow until metal ball is 5.5, then switch from air to nitrous oxide.
Recorder Readout
Noise Suppression - 3
Scale - 10X
Recorder Settings
0.5 inch/min.

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD No. 995
ANALYTICAL METHOD	ATOMIC ABSORPTION-ANALYSIS ALUMINUM IN ANTIHEMOPHILIC FACTOR

INTERPRETATION OF RESULTS

Since the absorption values obtained in this analysis are below 10%, there is no need to convert them to absorbance for calculation purposes. Use the absorption of the aluminum standard nearest the sample absorption and calculate the aluminum content of the sample by the following formula:

$$\frac{\text{Sample Absorption}}{\text{Standard Absorption}} \times \text{Al}_{\text{std}} \text{ (in ug/ml)} = \text{ug Al/ml}$$

In routine daily use of this procedure, the sample contains less aluminum per ml than the lowest aluminum standard (1 ug/ml) as is evident by the visual comparison on the strip chart recorder readout of the sample response to the standard response. To simplify the calculation, the sample response is visually compared to the standard response. The following example is presented to show how the final reported result is obtained. The reconstituted volume is also reported on the Analysis Report sheet.

Example for Spec. No. 3502:

Reconstituted volume = 50 ml.
Recorder response less than 1 ppm Al Std.
Report - Less than 50 ug Al. per vial
Reconstituted volume = 50 ml

If the Recorder response was more than 1 ppm and less than 2 ppm, the report should be:

Less than 100 ug Al. per vial
Reconstituted volume = 50 ml

REFERENCE

Perkin-Elmer Operator's Instructions

9/8/80
ml

AM-6600

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ARMOUR PHARMACEUTICAL COMPANY
QUALITY CONTROL DEPARTMENT
ANALYTICAL METHODS

METHOD NUMBER 1073

DETERMINATION OF THE HEPARIN CONTENT OF CRYOPRECIPITATED AHF

The following in vitro method is used in the determination of the Heparin content of cryoprecipitated AHF. The test is based on the in vitro inhibition by Heparin of coagulation time as measured by the activated partial thromboplastin time procedure.

I. Reagents and Equipment:

1. Heparin-potency 1000 units/ml. obtained from a reputable source.
2. Activated Partial Thromboplastin
3. Cryoprecipitated AHF (Lyophilized)
   (a) Heparin Containing
   (b) Non-Heparin Containing
4. Normal Control Plasma
5. A BBL Fibrometer for the determination of clotting times. This instrument should be equipped with an automatic pipette capable of dispensing 0.1 ml.
6. 0.025 molar calcium chloride
7. A Thermal Incubation Block (37°C.).

II. Procedure:

A. Reconstitution of Reagents

1. Reconstitute the normal coagulation control plasma (lyophilized) with 1 ml. of 3 (a) or 3(b).
2. Reconstitute the activated partial thromboplastin (lyophilized) with 2 ml. distilled water.
3. Reconstitute the AHF sample (lyophilized) with 25 ml. of distilled water or see appropriate supplement.

B. Dilution of Heparin for the Preparation of a Heparin Standard

1. Dilute the stock Heparin Solution of 1000 u/ml. with distilled water to provide the following concentrations, in 1 ml. of normal coagulation control plasma:

   0.25 u/ml., 0.30 u/ml., 0.35 u/ml., and 0.40 u/ml.

C. Determination of the Clotting Time of the Plasma with Heparin Added

1. Incubate the plasma, partial thromboplastin and 0.025 molar calcium chloride at 37°C. for five (5) minutes.

2. Using an automatic pipette, add 0.1 ml. of the plasma to a fibrometer reaction cup.

3. Add 0.1 ml. of partial thromboplastin to the plasma in the reaction cup and incubate exactly four (4) minutes.

4. At the end of four (4) minutes, add 0.1 ml. of calcium chloride and start timing of the clotting reaction.

5. Determine the clotting times for these Heparin containing plasma in 5 replicate determinations and plot on standard graph paper. Plot clotting time on the vertical axis and the Heparin content on the horizontal axis.

D. Determination of the Heparin Concentration in the AHF Sample

1. Reconstitute 1.0 ml. of normal coagulation control plasma with 1.0 ml. of the reconstituted AHF sample. Mix and incubate at 37°C. for five (5) minutes in a thermal block.

2. Determine the activated partial thromboplastin clotting times in 5 replicate determinations.

3. Calculate the Heparin concentrations of the AHF sample from the Heparin Standard and correct for dilution.

4. Express the Heparin concentration in units/ml.

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8/31/76

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD NO. 1257
ANALYTICAL METHOD	SOLUTION TIME FOR GENERATION II ANTIHEMOPHILIC FACTOR (A.H.F.)

DATE:	SUPERSEDES:	PREPARED BY:
2/21/77	New	L. E. Caricofe

TEST SUMMARY

The purpose of this method is to determine the rate of reconstitution time of Generation II A.H.F.

COMMENTS

a. Safety Precautions: None.

b. General Precautions: Care should be exercised when swirling the vials so as to minimize the foaming.

MATERIALS FOR TESTING

1. Sterile water for injection, U.S.P. (30 ml./vial)
2. Double-ended reconstitution needle
3. Stop watch or timer
4. Water bath at 37°C.

TEST PROCEDURE

1. Warm both the 30 ml. diluent of sterile water for injection and vial of Generation II A.H.F. (unopened vials) to 37°C. in a water bath (not less than 15 minutes).

2. Remove caps from both vials to expose the central portion of rubber stoppers.

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Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD NO. 1257
ANALYTICAL METHOD	SOLUTION TIME FOR GENERATION II ANTIHEMOPHILIC FACTOR (A.H.F.)

3. Insert one end of the double-ended needle into the rubber stopper of the diluent vial. Invert the diluent vial and insert the other end of the double-ended needle into the rubber stopper of the A.H.F. vial. Allow the diluent to be drawn into the A.H.F. vial by vacuum and direct the stream over the surface of the cake.

4. Release the vacuum by removing the diluent vial from the double-ended needle; then remove the double-ended needle from the A.H.F. vial allowing the incoming airstream to agitate the vial contents. Start a stop-watch.

5. Permit the A.H.F. vial to sit at room temperature for 1 minute without any agitation. This permits thorough wetting of the A.H.F. cake and improves the rate of reconstitution.

6. Manually swirl the A.H.F. vial very gently (avoid foaming) to promote breakup of the cake. As more cake goes into solution, more rapid swirling is permissable to get the last few particles into solution (avoid foaming).

7. Discontinue assay when the A.H.F. is in solution. The A.H.F. must be in solution after 30 minutes of continual swirling at room temperature 20-37°C. Record reconstitution time.

INTERPRETATION OF RESULTS

Report the reconstitution time to the nearest minute.

REFERENCES

Memo to Mr. A. K. Roop from Dr. Fred Feldman dated January 18, 1977.

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD NO. 1301
ANALYTICAL METHOD	SODIUM AND POTASSIUM DETERMINATIONS Using the Instrumentation Laboratory Model 343 Flame Photometer

DATE:	SUPERSEDES:	PREPARED BY:
2/13/78	New	W. H. Osgood

TEST SUMMARY

The method quantitatively determines the concentration of sodium and potassium ions in samples using Flame Emission Photometry. The method uses Lithium as an internal standard and as a radiation buffer. In the latter role, it removes the variability of potassium determinations due to changing concentrations of sodium. The variation is due to energy transfers in the excited state of the ions. Lithium must be present for proper operation of the instrument. This method is applicable only to analyses done using the Instrumentation Laboratory Digital Flame Photometer Model #343.

COMMENTS

a. Safety Precautions: Normal precautions for instruments using compressed gases must be used. Since human blood products are used, care must be exercised to prevent the transmission of disease. The instrument must NOT be operated without the Flame Housing Cover in place.

b. General Precautions: The sample must have sodium and potassium levels in the range of the standards described. All glassware must be scrupulously clean to avoid variations due to alkali contamination.

c. Non-Standard Abbreviations: The following non-standard abbreviations are used

DD water = distilled deionized water

REASON FOR REVISION:

APPROVED BY:

GRO-C	March 28, 1978
GRO-C	3/28/78 3/30/78
GRO-C	4/3/78
GRO-C	4/6/78

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ARMO0000092_0096

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD No. 1301
ANALYTICAL METHOD	SODIUM AND POTASSIUM DETERMINATIONS Using the Instrumentation Laboratory Model 343 Flame Photometer

MATERIAL FOR TESTING

A. Equipment and Supplies
   1. Instrumentation Laboratory Digital Flame Photometer Model #343
   2. Compressed air (From a cylinder or air compressor, 25 p.s.i. minimum, preferred range 30 - 40 p.s.i.)
   3. Instrument Grade Propane. Use IL Cat. #57000 only. Do not substitute.
   4. Volumetric Glassware (Flasks & Pipets)
   5. Disposable beakers or medicine cups, approximately 30 ml. capacity

B. Reagents: Described in sections where required.

C. Standards: Described in sections where required.

TEST PROCEDURE

This is divided into four sections. Refer to the proper section for the technique required. For all A.H.F. & N.S.A. samples, use Section I only. Section II and III are to be used only with the approval of the manager of the control laboratory. For all other samples, use the proper supplement to this method.

I. Manual Dilution with direct readout
II. Manual Dilution with indirect readout
III. Automatic Dilution with direct readout
IV. Instrument Operation

I. Manual Dilution with Direct Results

CAUTION: It is imperative that the standards and samples are prepared using the same Lithium Internal Standard. If two different solutions are used, the results obtained will be meaningless.

A. Reagents

1. Lithium Internal Standard: Dilute 20 ml. of Lithium Stock concentrate (IL Cat. #35003) to one liter. Mix well. Substitution is not recommended since this solution contains a nonionic surfactant.

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD No. 1301
ANALYTICAL METHOD	SODIUM AND POTASSIUM DETERMINATION Using the Instrumentation Laboratory Model 343 Flame Photometer

B. Standards

Standards can be prepared in two ways. The first method is desirable and the second to be used only when absolutely necessary.

1. Preferred Standards: Use IL standards of the appropriate values. For sodium analysis of A.H.F. & N.S.A., use 140 meq.Na/l and 5 meq. K/liter (Catalog #35140) and 100 meq. Na/l and 100 meq K/l (IL Cat. #35100) as the midpoint standard.

   For potassium analysis, use the levels that are close to the values of potassium to be measured. Since the expected values of potassium are less than 5 meq/l, use IL Cat. #35140 as the high standard (5 meq. K/l) and IL cat. #35120 as the midpoint standard.

   To prepare the standards for use, pipet 1.0 ml. of the standard into a 200 ml. volumetric flask. Pipet 50.0 ml. of Lithium Internal Standard into the same flask. Dilute to the mark with DD water and mix well. A zero standard is required which is prepared by pipeting 50.0 ml. of Lithium Internal Standard into a 200 ml. volumetric flask and diluting to the mark with DD water. Mix well.

2. Backup Standards: Use reagent grade (or better) chemicals.
   a. 140 meq Na/liter. Weigh out accurately approximately 14.0 meq of sodium chloride (819 milligrams). Transfer to a 100 ml. volumetric flask. Dissolve in DD water and dilute to the mark. For use, pipet 1.0 ml. into a 200 ml. volumetric flask. Pipet 50.0 ml. of Lithium Internal Standard into the same flask. Dilute to the mark with DD water and mix well. If the weight used is not exact, calculate the true value of the standard as below

$$\text{True value (meq/l.)} = \frac{\text{weight used in milligrams}}{5.85}$$

   b. 100 meq Na/liter. Weigh out accurately approximately 10.0 meq. of sodium chloride (585 milligrams). Transfer to a 100 ml. volumetric flask. Proceed as above (2.a.) starting with "Dissolve in DD water...".

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD No. 1301
ANALYTICAL METHOD	SODIUM AND POTASSIUM DETERMINATIONS Using the Instrumentation Laboratory Model 343 Flame Photometer

B. Standards (Continued.)

c. 5 meq. K/liter. Weigh out accurately approximately 5 meq. of potassium chloride (373 milligrams). Transfer to a 1000 ml. volumetric flask. Dissolve in DD water and dilute to the mark. For use, pipet 1.0 ml. into a 200 ml. volumetric flask. Pipet 50.0 ml. of Lithium Internal Standard into the same flask. Dilute to the mark with DD water and mix well. If the weight used is not exact calculate the true value of the standard as below

$$\text{True value (meq/l)} = \frac{\text{weight used}}{74.6}$$

d. 2 meq. K/liter Weigh out accurately approximately 2 meq. of potassium chloride (149.2 mg). Transfer to a 1000 ml volumetric flask. Proceed as above (2.c.) starting with "Dissolve in DD water...".

C. Sample Preparation

1. For A.H.F. samples: Reconstitute the samples as specified in the specification. Use the reconstituted solution and follow the method below for N.S.A. samples.

2. For N.S.A. samples: Pipet accurately and precisely 1.0 ml. of the sample into a 200 ml. volumetric flask. Pipet 50.0 ml. of Lithium Internal Standard into the flask. Dilute to the mark with DD water and mix well.

3. For all other samples, see the supplement or seek qualified assistance.

D. Method of Operation - Refer to Figures 1, 2, 3 & 4.

1. All solutions to be aspirated into the instrument are put in 30 ml. disposable beakers to the same level, 3/16" from the top. During aspiration, the beakers are placed on an inverted 50 ml. beaker under the aspirating tube.

2. Refer to Section IV for Instrument Operation and turn on.

3. Place a sample cup containing DD water under the aspirating tube and allow it to draw for several seconds.

4. Remove this and wipe the aspirating tube.

NOTE: The following is written for sodium determinations. Using the potassium standards and making corrections for the controls, the procedure is applicable to potassium determinations.

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Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD No. 1301
ANALYTICAL METHOD	SODIUM AND POTASSIUM DETERMINATIONS Using the Instrumentation Laboratory Model 343 Flame Photometer

5. Place the zero sodium standard under the aspirating tube and allow it to draw.

6. After the digital displays and the meter have settled, set the Lithium Response Meter Needle (No. 2 on figure 2) at the triangle (see figure 4) using the Lithium Set Control (No. 1 on figure 2).

7. Adjust the Sodium Digital Concentration Display (No. 4 on figure 2) to 000.0 using the Sodium Zero Control (No. 16 on figure 3). Operate the Sodium Decimal Position Button (No. 5 on figure 2) to place the decimal point at the proper position (000.0).

8. Remove the Zero Standard Solution and wipe the aspirating tube. Allow air to draw for about 10 seconds.

9. Place the highest level of sodium standard under the the aspirating tube and allow it to draw.

10. After the digital displays and the meter have settled, adjust the Sodium Digital concentration display to the value of the standard using the Sodium Balance Control (No. 3 on figure 2). If the standard used is the Cat. #35140, the display would be adjusted to 140.0.

11. Remove the standard and allow air to aspirate for approximately 5 seconds.

12. Place DD water briefly under the aspirating tube ($\sim$ 2 seconds) and then allow air to aspirate for approximately 5 seconds.

13. Place the midrange standard under the aspirating tube and allow it to draw.

14. After the displays and meter have settled, record the value from the Sodium Digital Concentration Display as milliequivalents sodium per liter.

    NOTE: The value should be within approximately $\pm$ 1 meq. of the prepared (or label) value. If it is not, recheck the higher level standard and reset if necessary. If this does not correct the problem, prepare new dilutions of the standards. If this does not correct the problem, seek qualified assistance.

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Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD No. 1301 SODIUM AND POTASSIUM DETERMINATIONS Using the Instrumentation Laboratory Model 343 Flame Photometer
ANALYTICAL METHOD	

15. Remove the solution from under the aspirating tube and allow air to aspirate for approximately 5 seconds. Place DD water briefly under the aspirating tube (~ 2 seconds) and then allow air to aspirate for approximately 5 seconds.

16. Place the sample under the aspirating tube and allow it to draw.

17. After the digital displays and meter have settled, record the value from the Sodium Digital Concentration Display as milliequivalents sodium per liter.

18. Repeat steps 15 through 17 once. Average the values obtained and report the result as milliequivalents per liter.

19. To run the next sample repeat steps 15 through 18.

20. After approximately four samples, repeat steps 8 through 14 but do not adjust the displays. If the high level standard has drifted more than approximately ± 0.8 meq./l., reset it and repeat the samples previously run.

21. To shut down the instrument, see Section IV.

II. Manual Dilution with Indirect Results

A. Reagents: Same as I

B. Standards: Same as I

C. Sample Preparation: Same as I

D. Method of Operation: The operation is identical except as noted below

Step 10. Adjust the display to an arbitrary number and record it.
Step 14. Record the value of the Sodium Digital Concentration display.
Step 17. Record the value of the Sodium Digital Concentration display.
Step 22. See Interpretation of Results for calculations.

ADI -6000

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD No. 1301
ANALYTICAL METHOD	SODIUM AND POTASSIUM DETERMINATIONS Using the Instrumentation Laboratory Model 343 Flame Photometer

III. Automatic Dilution with Direct Results

A. Reagents:

1. Lithium Stock Concentrate: Use IL Cat #35003 only. Use as received.

B. Standards:

1. Sodium/Potassium Standards: Use only the IL Calibration Standards listed below.
   a. Sodium Analysis: Use Sodium Standards 140 meq./l. (Cat. #35140) and 100 meq./l. (Cat. #35100)

   b. Potassium Analysis: Use Potassium Standard 5 meq./l. (Cat. #35140) and 2 meq./l. (Cat. # 35120)

C. Sample Preparation: NOTE: N.S.A. can not be reliably performed using this method.

1. A.H.F. Samples: Reconstitute according to the specification and then use the resulting solution.

2. Human Serum and related samples: Use as received. If any cells or other material are present, they must not be allowed to enter the dilutor. If the sample is hemolyzed, the results may differ from the true value.

3. Other samples: Seek qualified assistance.

D. Method of Operation - Refer to Figures 1, 2, 3, & 4.

1. Refer to Section IV for instrument start up and ignition.

2. Ascertain that the bottles containing diluent water, rinse solution and Lithium Stock Concentrate have sufficient volume. There should be at least 5 liters of distilled water in the diluent reservoir.

3. Turn on the dilutor and make certain that the sample cup is draining properly.

4. Leave the sampling tube in the flushing cup for about 15 seconds.

5. Remove the sampling tube and wipe it using a lint free wiper (i.e. Kim-Wipes). Allow it to remain out of solution for 10 seconds.

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD No. 1301 SODIUM AND POTASSIUM DETERMINATIONS Using the Instrumentation Laboratory Model 343 Flame Photometer
ANALYTICAL METHOD	

6. Place the sampling tube into a sample cup containing DD water. After the digital displays and Lithium meter have settled, set the Lithium Response Meter needle (No. 2 on figure 2) at the triangle (See figure 4) using the Lithium Set Control (No. 1 on figure 2).

7. Adjust the Sodium Digital Concentration Display to 000.0 (No. 4 in figure 2) using the Sodium Zero Control (No. 16 in figure 3). Operate the Sodium Decimal Position Button (No. 5 on figure 2) to place the decimal point at the proper position.

8. Adjust the Potassium Digital Concentration Display (No. 7 on figure 2) to 00.00 using the Potassium Zero Control (No. 17 on figure 3). Operate the Potassium Decimal Position Button (No. 8 on figure 2) to place the decimal point at the proper position.

9. Remove the sampling tube and wipe using a lint-free wiper and allow it to remain out of solution for about 10 seconds.

10. Place the sampling tube into the standard solution. After the Lithium meter and displays are settled, adjust the Sodium and Potassium Concentration Digital Displays to their proper values (as dictated by the standard solution being used) using the Sodium Balance Control (No. 3 on figure 2) and Potassium Balance Control (No. 6 on figure 2). For example, if a sodium standard of 140 meq/liter is being used, adjust the sodium display to 140.0.

11. Remove the sampling tube from the standard and wipe it, using a lint-free wiper. Allow it to remain out of solution for 10 seconds.

12. Place the sampling tube in the flushing cup and leave it there for 10 seconds. Remove it, wipe with a lint-free wiper and allow it to remain out of solution for 10 seconds.

13. Place the sampling tube into the midrange sodium standard. After the displays have settled, record the value from the display.

    NOTE: The value for the sodium standard should be within ± 1 meq/liter of the label value. If it is not, recheck the higher level standard and reset if necessary. If this does not correct the problem, seek qualified assistance.

    Repeat the procedure for the midrange Potassium Standard (allowing an air & rinse flush between standards as per steps 11 & 12). It should be within ± 0.1 meq./liter.

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD No. 1301
ANALYTICAL METHOD	SODIUM AND POTASSIUM DETERMINATIONS Using the Instrumentation Laboratory Model 343 Flame Photometer

14. Remove the sampling tube and wipe using a lint-free wiper. Allow it to remain out of solution for about 10 seconds.

15. Place the sampling tube in the flushing cup and leave it there for 10 seconds. Remove it, wipe with a lint-free wiper and allow it to remain out of solution for 10 seconds.

16. Place the sampling tube into the sample. After the displays have settled, record the values from the display.

17. Repeat steps 14 through 16 once. Average the results obtained and report these results.

18. To run the next sample, repeat steps 14 through 17.

19. After approximately four samples, repeat steps 9 through 13 but do not adjust the displays. If the high level standard has drifted more than approximately ± 0.8 meq. Na/l (or 0.08 meq. K/liter). reset it and repeat the samples previously run.

20. To shut down the instrument, see Section IV.

IV. Instrument Operation

A. Turn On Ignition: The flame is automatically ignited when sufficient gas and air pressures are available. Do not operate the instrument without the Flame Housing Cover in place.

1. Open the Propane Gas Valve at the top of the fuel cylinder (No. 18 on figure 3) 2.5 counterclockwise turns. A stop should be felt at the end of the rotation.

2. Open the main valve on the cylinder of compressed air. Adjust the regulator to 30 - 40 psi. Open valve at outlet of regulator (if so equipped).

3. Press the POWER button. The FLAME ON light should come on within 10 seconds. During this time, a clicking noise will be heard.

4. If the NO AIR and/or NO GAS lights turn on, this problem must be corrected. Consult the instruction manual for gas cylinder replacement. For other problems, seek qualified assistance.

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD No. 1301
ANALYTICAL METHOD	SODIUM AND POTASSIUM DETERMINATIONS Using the Instrumentation Laboratory Model 343 Flame Photometer

5. Allow the instrument to warm up for approximately 20 minutes.

6. At the end of this period, place the sample cup in position under the aspirating tube. (see figure I). Turn on the dilutor and make certain the drain is operating. Make certain there is at least 5 liters of water in the reservoir.

7. Place the sampling tube in the flushing cup.

8. After 10 minutes, check the atomizing chamber. It should be uniformly wet without a large amount of droplets visible. If droplets are visible, seek qualified assistance.

9. For Manual Dilution Assays
   a. Remove the sampling cup and store in an unused area after turning off the dilutor.
   b. Place a diluted sample under the aspirating tube for approximately 2 minutes. The atomizing chamber should still be uniformly wetted. If so, proceed as under Section I (or II).

10. For Automatic Dilution Assays
    a. Place the sampling tube into a sample for approximately two minutes. The atomizing chamber should still be uniformly wetted. If so, proceed as under Section III.

B. Shutdown

1. When running an automatic dilution assay, draw cleaning agent (IL Cat #33104) through the dilutor for 30 seconds to clean proteins and other material from the pump tubing.

2. For all assays, place the sampling tube in the flushing cup and allow it to remain there. Place the sample cup in position under the aspirating tube. Turn on the dilutor (if not on) and check for proper drain operation.

3. After approximately five minutes, turn off the dilutor. Remove the sample cup from under the aspirating tube and place in an unused location.

4. When the atomizing chamber is dry (5-10 minutes), turn off valve at the top of the propane cylinder. (2.5 turns clockwise)

5. After the FLAME ON lamp turns off, close the compressed air supply valve off.

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Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD No. 1301
ANALYTICAL METHOD	SODIUM AND POTASSIUM DETERMINATIONS Using the Instrumentation Laboratory Model 343 Flame Photometer

B. Shutdown Continued.

6. Turn off the power by pushing the POWER button. After 30 seconds, the instrument will shut down.

7. Release the air pressure by removing and then replacing the fitting on the air supply hose.

C. Preventive and Corrective Maintenance.

1. Refer to the Operation Manual and/or seek qualified assistance.

INTERPRETATION OF RESULTS

A. Calculations for direct results for A.H.F. and N.S.A. samples and all automatic dilution samples.

No calculations are required.

B. Calculations for Indirect Results

1. Using the data from the standards, draw a standard curve by plotting the Display value (y) versus the concentration of the standards (before dilution) ($\bar{x}$).

2. Using the standard curve, determine the values of the samples. Average the replicates for each sample and report those results.

3. The proper results will be obtained only when the procedure is followed exactly as written.

REFERENCES

1. "Operation Manual for the Model 343, Digital Flame Photometer" Instrumentation Laboratory, Inc. Lexington, Mass. (1975)
2. Armour Notebook #K901.

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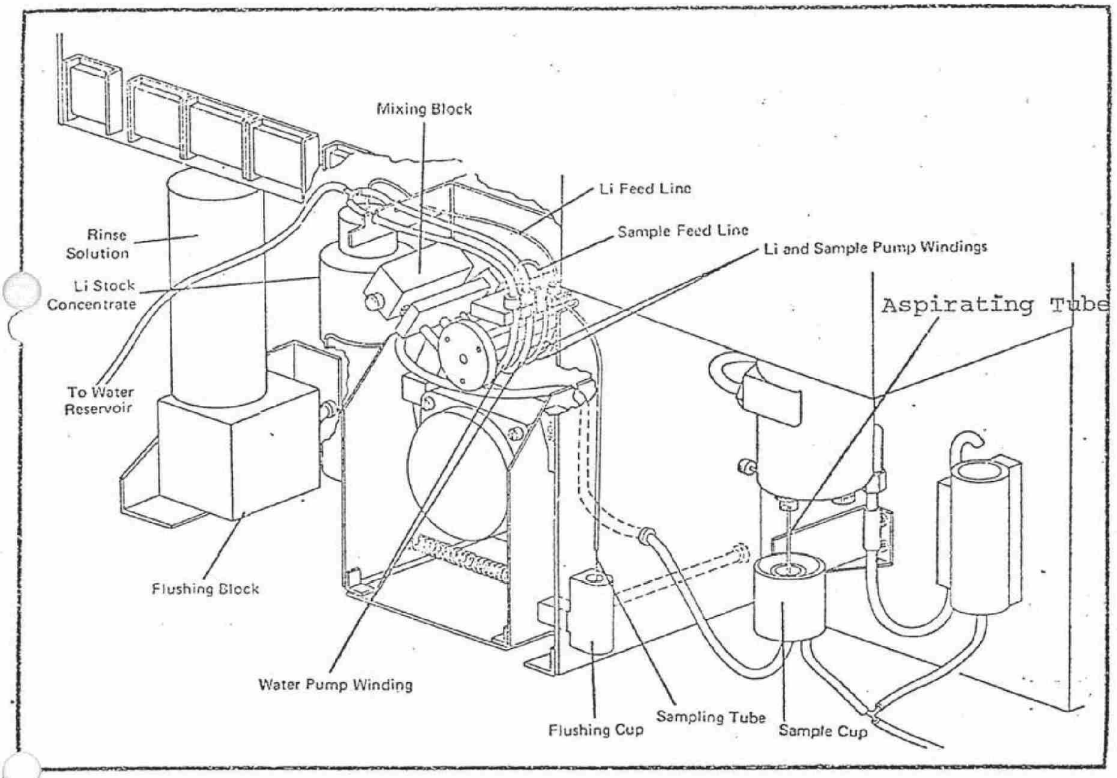

Figure 1

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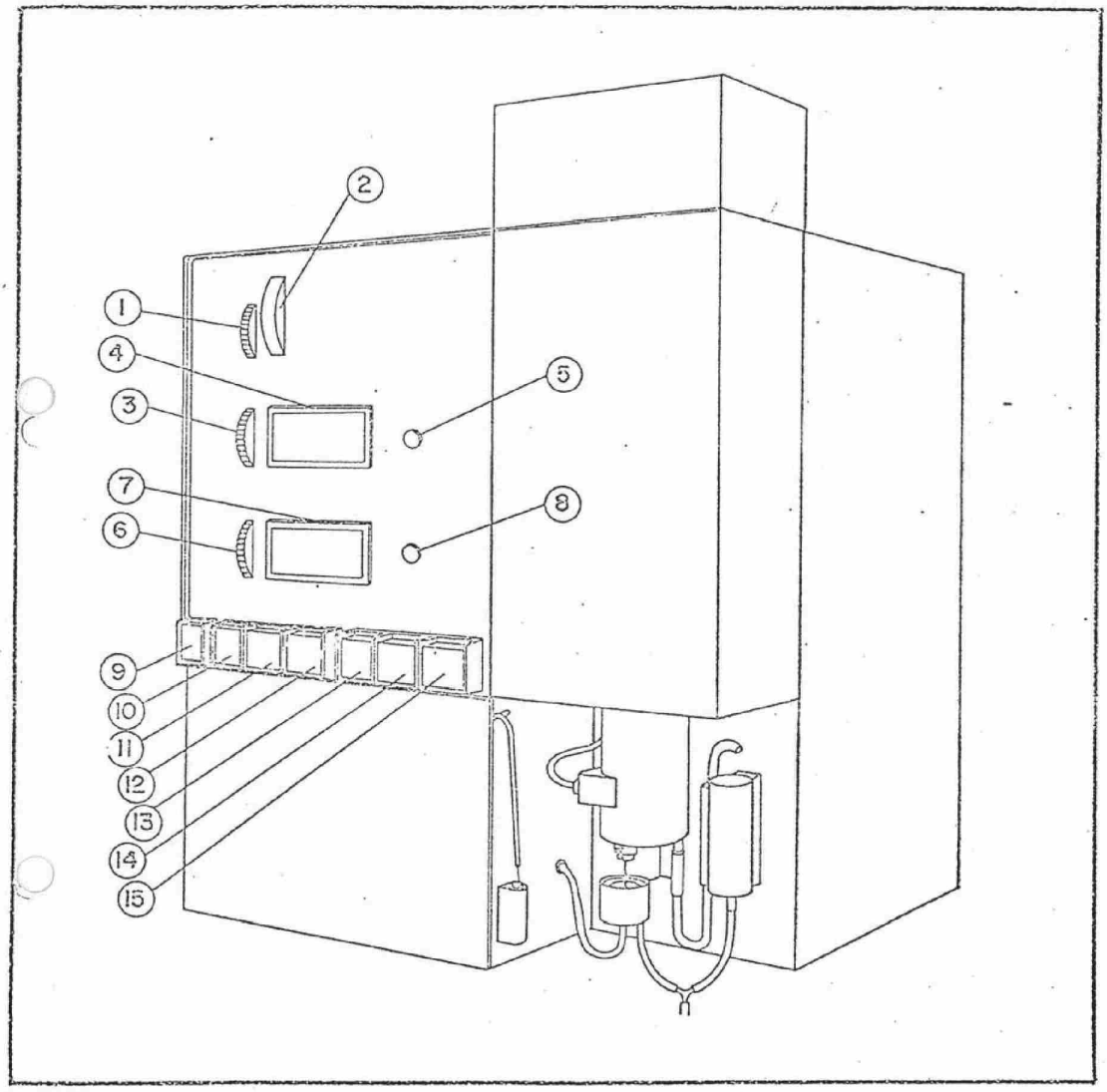

Figure 2

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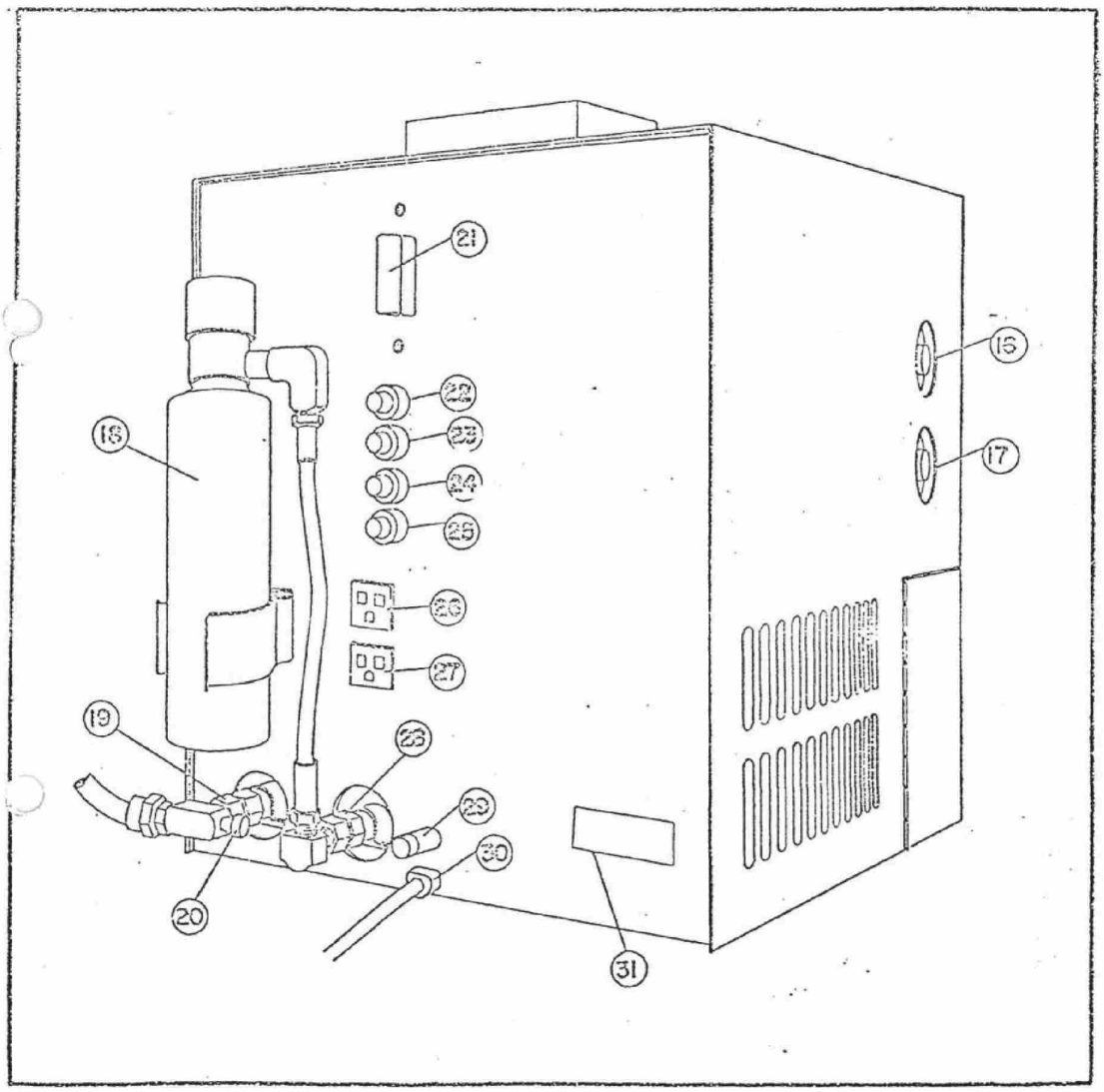

Figure 3

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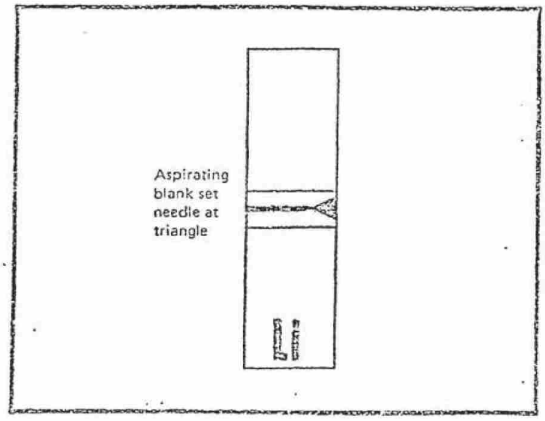

Figure 4

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD NO. 1301 Supplement 2
ANALYTICAL METHOD	SODIUM & POTASSIUM DETERMINATIONS AHF SAMPLES WITH VARIED RECONSTITUTION VOLUMES

DATE:	SUPERSEDES:	PREPARED BY:
4/8/78	New	W. H. Osgood

This supplement describes the sample preparation required for single fill AHF samples reconstituted to 10 ml and double fill AHF samples reconstituted to 20 ml.

Sample Preparation

The sample is reconstituted to the volume requested. Pipet a 2.0 ml aliquot of this into 5 ml volumetric flask. Dilute to the mark with DD water and mix well.

Pipet 1.0 ml of the diluted sample into 200 ml volumetric flask. Pipet 50.0 ml of Lithium Internal Standard into the same flask. Dilute to the mark with DD water and mix.

Method of Operation

Refer to the method.

Interpretation of Results

Due to the additional 4 to 10 dilution, the result obtained from the display must be multiplied by 2.5 to obtain meqNa/l in the original sample.

REASON FOR REVISION:	APPROVED BY:
	GRO-C May 3, 1978 GRO-C 5-8-78 5-12-78 GRO-C 5/15/78 GRO-C 5/18/78

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Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD NO. 1344
ANALYTICAL METHOD	DETERMINATION OF FIBRINOGEN

DATE	SUPERSEDES:	PREPARED BY:
3/21/79	New	A. K. Roop

TEST SUMMARY

An aliquot of the sample solution is reacted with thrombin reagent and the time required for clotting is measured using a Fibrometer. Final concentration of fibrinogen is determined by reference to a standard curve prepared using reference fibrinogen.

COMMENTS

A. Safety Precautions

General laboratory safety conditions prevail.

B. General Precautions

Any samples from human blood, although nonreactive when tested for hepatitis associated antigen, may still present a risk of transmitting viral hepatitis, as no completely reliable laboratory test is yet available for hepatitis virus.

MATERIALS FOR TESTING

1. Fibrinogen Reference Standard

Data - FI Fibrinogen calibration reference standard is purchased from Dade Reagents, Inc., Miami, Florida (Scientific Products, distributor). Reconstitute the lyophilized contents of one vial with 1 ml of distilled water. A fibrinogen reference material from another source may be substituted.

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD No. 1344
ANALYTICAL METHOD	DETERMINATION OF FIBRINOGEN

MATERIAL FOR TESTING

(Cont'd.)

2. Bovine Topical Thrombin (Parke-Davis #4-2076-1)

   Reconstitute the contents of 1 vial (1000 NIH units per vial) with 20 ml of 0.9% saline. Divide the solution into 4 ml aliquots, transfer to appropriate size vials, stopper, and store frozen at -20°C. During the assay, keep the thawed working solution in an ice bath. Thrombin obtained from a supplier, other than Parke-Davis, may be substituted.

3. Owren's Veronal Buffer

   Into a 1 liter beaker containing about 800 ml of distilled water, add 5.855 g of sodium barbital and 7.306 g of reagent grade sodium chloride. Mix until the reagents have dissolved. Using a pH meter measure the pH of the solution and adjust to pH 7.35 using 1N hydrochloric acid. Quantitatively transfer the solution to a 1 liter volumetric flask and dilute to volume with distilled water.

4. Saline Solution

   Dissolve 9.0 g of reagent grade sodium chloride in sufficient distilled water to make 1000 ml.

5. Fibro System consisting of a Fibrometer Precision Coagulation Timer, a Thermal Prep Block and an Automatic Electric Pipette. The Fibro System may be purchased from BBL, Cockeysville, Maryland. An equivalent unit may be substituted.

TEST PROCEDURE

1. Using the Owren's Veronal buffer as diluent, prepare each of the following Reference Standard dilutions just prior to use. Do not prepare all dilutions simultaneously. Assay each dilution in duplicate.

- continued on next page -

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Armour Pharmaceutical Company
QUALITY STANDARDS
Kankakee, Illinois

APC METHOD No. 1344

ANALYTICAL METHOD

DETERMINATION OF FIBRINOGEN

- continued from page 2 -

1:5 - 0.2 ml Fibrinogen Ref. Std. plus 0.8 ml Veronal Buffer
1:10 - 0.1 ml Fibrinogen Ref. Std. plus 0.9 ml Veronal Buffer
1:15 - 0.1 ml Fibrinogen Ref. Std. plus 1.4 ml Veronal Buffer
1:20 - 0.1 ml Fibrinogen Ref. Std. plus 1.9 ml Veronal Buffer
1:30 - 0.1 ml Fibrinogen Ref. Std. plus 2.9 ml Veronal Buffer

NOTE: Use automatic 2-step pipette gun for accurate 100 and 200 µl volumes.

2. Incubate 0.2 ml of the fibrinogen standard dilution (or sample dilution to be tested) at 37°C for 2 minutes.

3. Add 0.1 ml of thrombin reagent and start the timer to determine clotting time.

4. Average the duplicate determinations to determine the clotting time for the dilution being tested. The standard deviation for the averaged value should be ± 0.5 seconds.

5. Based on the expected amount of fibrinogen in the sample prepare three dilutions of the sample that will result in clotting times within the range of clotting times obtained for the Reference Standard. Use the Veronal Buffer for all dilutions and assay duplicate aliquots from each dilution by repeating Steps 2, 3, and 4.

INTERPRETATION OF RESULTS

1. Plot the Fibrinogen Reference Standard concentration vs. clotting time on log - log paper and connect the points with the best straight line or use a linear regression analysis.

2. By reference to the standard curve calculate the fibrinogen in the sample dilution; report as mg fibrinogen per ml solution.

REFERENCES

Data - FI Fibrinogen Determination, Dade Diagnostics, Inc., 1851 Delaware Parkway, Miami, Florida

sd
2/21/79

AP000693

ARMOUR000900

ARMO0000092_0114

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD NO. 1402
ANALYTICAL METHOD	TOTAL CITRATES IN ANTIHEMOPHILIC FACTOR

DATE	SUPERSEDES:	PREPARED BY:
9/5/80	New	L. Cotter

TEST SUMMARY

The sample is diluted with triple distilled water and the citrate is determined by an enzymatic reaction. The reaction is monitored by observing the change in absorbance at 340 nm due to the oxidation of NADH (β-nicotinamide adenine dinucleotide, reduced form). The citrate is calculated from the extinction coefficient of the NADH since the amount of NADH is stoichiometric with the amount of citrate.

COMMENTS

a. Safety Precautions: General laboratory safety conditions prevail.

b. General Precautions:
   1. Only free citrates are determined, not esterified citrates.
   2. Oxaloacetate and pyruvate are substrates for the indicator reactions, therefore the sample must be relatively free of these compounds. Trace levels can be tolerated only if they are allowed to react completely before the citrate lyase addition.

NOTE: The method is written in two sections for using either a purchased kit (Section I) or preparing the reagents in laboratory (Section II). It is preferable to use the kit.

REASON FOR REVISION:	APPROVED BY:
	GRO-C 9-3-80 GRO-C 9-3-80 GRO-C 9-3-80 GRO-C 9-4-80 GRO-C 9/5/80 AP000694

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD No. 1402
ANALYTICAL METHOD	TOTAL CITRATES IN ANTIHEMOPHILIC FACTOR

I. DETERMINATION USING THE CITRIC ACID KIT
(Boehringer Mannheim Cat. No. 139076)

MATERIALS FOR TESTING

A. Equipment and Supplies

1. A precision spectrophotometer with an output linear in absorbance capable of operating at 340 nm and using 1 cm cuvettes (nondisposable). The Cary 15 is a suitable unit.

2. Volumetric Glassware

3. Optional: Eppendorf style automatic pipets for measuring 20 µl, 100 µl, and 200 µl.

B. Reagents

1. All required reagents are contained in the kit. Reconstitute the vials as needed. Refer to the kit instructions for the proper volumes. Use distilled water and pipets. The solutions are stable as described in the instructions and must be stored as specified.

C. Standards

Standards are not required, but can be run if desired to confirm the analysis. See Method 1390.

D. Sample Preparation

Reconstitute the sample as directed on label or specification. After the sample is in solution, dilute 1.0 ml to 50 ml in a volumetric flask using triple distilled water. The sample is to be run in duplicate.

TEST PROCEDURE

A. Set the spectrophotometer to 340 nm and adjust the unit to zero absorbance with nothing in either the sample or reference beams.

B. Blank Determination

1. Into a clean, dry 1 cm cuvette, pipet 2.0 ml distilled water followed by 1.0 ml from bottle #1. Mix well.

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD No. 1402
ANALYTICAL METHOD	TOTAL CITRATES IN ANTIHEMOPHILIC FACTOR

TEST PROCEDURE (Con't.)

B. (Con't.)

2. Place the cuvette in the spectrophotometer and determine the absorbance, A_1, when stable.

3. Pipet into the cuvette 20 µl (0.020 ml) from bottle #2. Mix well. Follow the change in absorbance until it has reached equilibrium (i.e., the reaction is complete). Record A_2.

4. Repeat steps 1 through 3 for a second blank.

C. Sample Determination

1. Into a clean, dry 1 cm cuvette, pipet 1.8 ml distilled water followed by 1.0 ml from bottle #1.

2. Pipet in 200 µl (0.20 ml) of the diluted sample or standard. Mix well.

3. Place the cuvette in the spectrophotometer and determine the absorbance, A_1, when stable.

4. Pipet into the cuvette 20 µl (0.020 ml) from bottle #2. Mix well. Follow the change in absorbance until it has reached equilibrium (i.e., the reaction is complete). Record A_2.

5. Repeat steps 1 through 4 for a duplicate assay using a second diluted sample. Each lot is to be run in duplicate using two different diluted samples.

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD No. 1402
ANALYTICAL METHOD	TOTAL CITRATES IN ANTIHEMOPHILIC FACTOR

INTERPRETATION OF RESULTS

For both the blank and the sample, determine the ΔA as

$$\Delta A = A_1 - A_2$$

Determine the average ΔA for the blank and calculate the ΔA_T for the sample as

$$\Delta A_T = \Delta A_{sample} - \Delta A_{blank}$$

$$\text{Citrate, g/L} = \frac{3.02 \times 191.1}{6.3 \times 1.0 \times 0.2 \times 1000} \times \Delta A_T \times \text{Dilution Factor}$$

$$\text{Citrate, mm/L} = \frac{3.02}{6.3 \times 1.0 \times 0.2} \times \Delta A_T \times \text{Dilution Factor}$$

II. DETERMINATION USING REAGENTS PREPARED IN LABORATORY

MATERIALS FOR TESTING

A. Equipment and Supplies -- (Same as Section I)

B. Reagents

Use reagent grade chemicals or better. The term water means the use of distilled water.

1. TEA Buffer with Zn, 0.1M, pH = 7.8: Weigh out 14.9 g of triethanolamine and dissolve in approximately 800 ml water. Adjust the pH to 7.8 ± 0.05 using dilute hydrochloric acid (1N or 10%). Add 28 mg of zinc chloride and mix well. Dilute to 1000 ml and mix.

2. NADH, 6mM: Weigh out 25 mg of β-Nicotinamide adenine dinucleotide, reduced, disodium salt (Boehringer Mannheim Cat. No. 107727 or equivalent) and 50 mg of sodium bicarbonate into a 5 ml volumetric flask. Dissolve the solids in water and dilute to the mark. Mix well. Kept refrigerated (2-8°C), this reagent is stable for about 1 day. Make fresh daily as needed. (Keep protected from light).

AP000697

DI-6600

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD No. 1402
ANALYTICAL METHOD	TOTAL CITRATES IN ANTIHEMOPHILIC FACTOR

MATERIALS FOR TESTING (Con't.)

B. Reagents (Con't.)

3. Dehydrogenase mixture: Pipet 0.1 ml of malate dehydrogenase (6000 units/ml, Boehringer Mannheim Cat. No. 127248 or equivalent) and 0.5 ml of lactate dehydrogenase (5500 units/ml, Boehringer Mannheim Cat. No. 127221 or equivalent) into a small container. Add 0.4 ml of TEA Buffer (1 above). This solution is stable 3 days if refrigerated.

4. Citrate Lyase: Use Boehringer Mannheim Cat. No. 103365. Dissolve 21 mg (± 0.5) of lyophilizate (an amount equivalent to approximately 5 mg of enzyme protein) in 1 ml ice-cold distilled water. The solution is stable for 3 days when refrigerated, or two weeks if frozen.

C. Sample Preparation -- (Same as Section I)

TEST PROCEDURE

A. Set the spectrophotometer to 340 nm and adjust the unit to zero absorbance with nothing in either the sample or reference beams.

B. Blank Determination

1. Into a clean, dry cuvette, pipet the reagents below in order:

2.9 ml of TEA Buffer
100 µl NADH
20 µl Dehydrogenase mixture
200 µl distilled water

2. Place the cuvette in the spectrophotometer and determine the absorbance, A_1, when stable.

3. Pipet into the cuvette 20 µl (0.020 ml) of the citrate lyase and mix well. Follow the change in absorbance until it has reached equilibrium (i.e., the reaction is complete). Record A_2.

4. Repeat steps 1 through 3 for a second blank determination.

AP000698

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD No. 1402
ANALYTICAL METHOD	TOTAL CITRATES IN ANTIHEMOPHILIC FACTOR

TEST PROCEDURE (Con't.)

C. Sample Determination

1. Into a clean, dry cuvette, (nondisposable) pipet the following in order:

   2.9 ml TEA Buffer
   100 µl NADH
   20 µl Dehydrogenase mixture
   200 µl diluted sample (or standard)

2. Place the cuvette in the spectrophotometer and determine the absorbance, A_1, when stable.

3. Pipet into the cuvette 20 µl (0.020 ml) of the citrate lyase and mix well. Follow the change in absorbance until it has reached equilibrium (i.e., the reaction is complete). Record A_2.

4. Repeat steps 1 through 3 for duplicate assays. Each sample is to be assayed in duplicate using two different diluted samples.

INTERPRETATION OF RESULTS -- (Same as Section I)

REFERENCES

1. Natelson, et al "Fluorometry of Citrate in Serum with the use of Citrate (pro-3S) lyase", Clin Chem 21, 730-734 (1975)

2. Methods in Enzymology, J.M. Lowenstein, Ed. Vol XIII Academic Press, 1969, pages 517-518

3. Methods of Enzymatic Food Analysis, Boehringer Mannheim 76/77

4. Armour Methods 1303, 1390, 1392, and 1393

5. Armour Notebook K895, pages 290 and 291

6. Memo to J. P. Aldred on validation of enzymatic method using British Pharmacopeia method as referee

7/8/80
dw

AP000699

Armour Pharmaceutical Company
QUALITY STANDARDS
Kankakee, Illinois

APC METHOD NO. 1410

ANALYTICAL METHOD

RIAUSURE II ANTIBODY TO HEPATITIS B SURFACE ANTIGEN (RIAUSURE II-Electro-Nucleonics Laboratories, Inc.)

DATE:	SUPERSEDES:	PREPARED BY:
7/10/80	New	A. K. Roop

TEST SUMMARY

RIAUSURE II is a solid phase radioimmunoassay system for detection of HB_sAg which employs Iodine-125 labeled Antibody to Hepatitis B Surface Antigen (Goat) (Anti-HB_s) as the indicator. The test employs the "sandwich technique". Anti-HB_s (Goat) is coated to controlled pore glass (CPG) particles in tablet form (solid phase). Patient serum is added to the tube containing a CPG tablet. During incubation the tablet disintegrates. If Hepatitis B Surface Antigen is present in the serum tested it will combine with the antibody on the glass particles. After incubation, the serum is removed and the glass beads rinsed. Iodine-125 labeled antibody specific for HB_sAg is then added.

The labeled antibody combines with the antigen bound to the antibody on the glass particles forming the "sandwich". The ^{125}I labeled antibody is the indicator which is detected in a gamma counter. The radioimmunoassay for HB_sAg is a qualitative test for the presence of Hepatitis B Surface antigen in serum. In general, however, the greater the amount of HB_sAg in a sample, the greater the number of counts per minute.

COMMENTS

Safety Precautions:

1. Handle all reagents as if capable of transmitting hepatitis. Mouth pipetting of reagents and samples must be avoided. Rubber gloves and protective clothing should be worn.

REASON FOR REVISION:

APPROVED BY:

GRO-C	6-30-80
GRO-C	7-2-80
GRO-C	7-8-80
GRO-C	7-10-80

AP000700

-0509

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD No. 1410 RIAUSURE II ANTIBODY TO HEPATITIS B SURFACE ANTIGEN (RIAUSURE II-Electro-Nucleonics Laboratories, Inc.)
ANALYTICAL METHOD	

Safety Precautions (Continued)

2. All materials used in this assay including reagents and samples should be disposed of in a manner that will inactivate human hepatitis virus. The preferred method is autoclaving for 60 minutes at 121°C. The liquid waste may be decontaminated by addition of a mixture of 20 ml of formalin and 60 ml of acetic acid per liter or sodium hypochlorite (bleach) 2.5% in the final volume. The waste should be allowed to stand overnight to inactivate the virus before disposal.

3. Eating, storing or preparing of food, smoking or applying cosmetics is banned in all areas where radioactive materials are stored or used.

4. Direct contact with radioactive materials must be avoided by using protective laboratory coats, wearing disposable plastic or rubber gloves and employing safety pipettes.

5. All spills of radioactive materials must be reported to the person in charge and decontaminated immediately. Contaminated wastes should be discarded with solid radioactive waste.

6. Current Federal Nuclear Regulatory Commission regulations allow for disposal of small quantities of labeled material, ^{125}I as in this kit, via the normal sewer system. Disposal by flushing should be restricted to sinks in authorized areas and limited to a few microcuries per day with adequate water flow.

7. Sterilized solid waste may be disposed of by conventional means. Consult the applicable regulations of Title 10, Code of Federal Regulations, Part 20 and local or State regulations.

8. WARNING: All samples should be treated as if capable of transmitting hepatitis. The precautions given above should be strictly observed.

General Precautions:

1. All reagents should be brought to room temperature before use.

AP000701

DI-6600

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD No. 1410 RIAUSURE II ANTIBODY TO HEPATITIS B SURFACE ANTIGEN (RIAUSURE II-Electro-Nucleonics Laboratories, Inc.)
ANALYTICAL METHOD	

General Precautions (Continued)

2. Insufficiently clotted plasma specimens are often responsible for false positive reactions in the RIA assay due to the presence of fibrin strands. Samples which contain fibrin strands should be heated at 56°C. for 20 minutes, the fibrin clot sedimented by centrifugation at 10,000 x G and the supernatant used for testing.

3. For patients receiving heparin therapy, such as those undergoing renal dialysis, blood for HB_sAg screening should be drawn before therapy is started. Blood drawn after therapy has started may clot in the RIAUSURE reaction tube causing a spurious result.

4. If samples are to be stored, care should be taken to maintain sterility. Sodium azide, to a concentration of 0.1%, may be added to preserve sterility. Store at 2° to 8°C.

5. Samples contaminated with microorganisms may also be unsuitable; care should be taken to preserve the sterility of samples.

6. A sample is non-reactive for HB_sAg if the ratio of the counts per minute (CPM) divided by the mean CPM of the negative control does not exceed an established cutoff ratio. Such a serum may considered to be non-reactive for Hepatitis B Surface Antigen and need not be tested further.

7. A serum is reactive for HB_sAg if the ratio exceeds the established cutoff. Because of the greater immunologic sensitivity of radioimmunoassay, false positive reactions are a possibility. For this reason, each reactive serum must be confirmed as positive for Hepatitis B Surface Antigen by a specificity test. Two classes of false positive reactions occur: 1) a nonrepeatable positive, due to technical error in testing, such as inadequate washing; 2) a repeatable positive which cannot be confirmed by specificity testing. The presence of Hepatitis B Surface Antigen may be confirmed by a specificity test which uses human Antibody to Hepatitis B Surface Antigen to neutralize the antigen in the sample. A small number

- continued on next page -

AP000702

DI-6600

Armour Pharmaceutical Company QUALITY STANDARDS Kenkakee, Illinois	APC METHOD No. 1410 RIAUSURE II ANTIBODY TO HEPATITIS B SURFACE ANTIGEN (RIAUSURE II-Electro-Nucleonics Laboratories, Inc.)
ANALYTICAL METHOD	

General Precautions

7. (Continued from preceding page)

of samples--usually less than 1 out of 10,000--will be repeatably positive but not confirmed by the specificity test. A reactive sample may be confirmed by a less sensitive test such as Counterelectrophoresis (CEP), or Agar Gel Diffusion (ADG). However, if there is disagreement between the two tests, the RIA specificity test must be performed. A specificity test to confirm the presence of Hepatitis B Surface Antigen in serum is available from Electro-Nucleonics Laboratories, Inc.--RIA-FIRM™ Specificity Test for Hepatitis B Surface Antigen.

MATERIALS FOR TESTING

A. RIAUSURE II Test Kit (100 tests), List No. 3910

Kit Contains:

100 reaction tubes (5 cards of 20 tubes each) each containing a CPG tablet coated with Antibody to Hepatitis B Surface Antigen (Goat) and a magnetized ferrite rod as a mixing device. 1 vial (10 ml) Antibody to Hepatitis B Surface Antigen ^{125}I (Goat); each vial contains less than 10 microcuries; perservative 0.1% sodium azide.

1 vial (5 ml) Negative Control (Human serum non-reactive for HB_sAg); preservative 0.1% sodium azide.

1 vial (2 ml) Positive Control (Human serum positive for HB_sAg); preservative 0.1% sodium azide.

1 vial Buffer Powder. Dissolve the contents of the vial in 1 liter of distilled or deionized water. The dissolved buffer is a 0.01M phosphate buffer (Na_2HPO_4/NaH_2PO_4) containing 0.15M NaCl. The pH should be 7.4 ± 0.2 units.

B. Magnetic Agitating Table (MAT) consisting of a controller and a Reaction Table. The reaction table holds 100 tubes in cards. The principles of operation of the MAT are included in the operating instructions for the instrument.

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD No. 1410 RIAUSURE II ANTIBODY TO HEPATITIS B SURFACE ANTIGENT (RIAUSURE II-Electro-Nucleonics Laboratories, Inc.)
ANALYTICAL METHOD	

MATERIALS FOR TESTING (Continued)

C. Wash Systems

1. Single Wash Devices

a. A device for delivering 1.0 ml of Buffer Solution. A 2.0 ml Cornwall syringe is suitable.

b. A wash cannula for attachment to the delivery device.

c. An aspiration cannula for removal of buffer from the reaction tubes. A vacuum source for use with the cannula is required.

2. Multiple Wash Devices

a. A multi-wash unit which washes 10 reaction tubes at the same time. This must be used with an appropriate dispensing system capable of delivering 10 ml of buffer (1.0 ml per tube).

b. A multi-aspirator unit which aspirates 10 reaction tubes at one time. A vacuum source for the multi-aspirator is required.

NOTE: The aspiration cannula or multi-aspirator unit may be attached to a suitable vessel connected to a trap which in turn is connected to a vacuum source. The collection vessel should contain sufficient disinfectant solution to inactivate the Hepatitis virus such as sodium hypochlorite (2.5% solution final concentration).

D. Pipettes capable of accurately delivering 0.1 and/or 0.2 ml.

E. Wash dispensing devices, such as Repipet or Brewer automatic syringes.

F. Counting tubes for Gamma Counter.

G. Gamma Counter capable of counting the weak gamma photon of ^{125}Iodine. (It is recommended that the counter efficiency be 50% or greater.)

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD No. 1410
ANALYTICAL METHOD	RIAUSURE II ANTIBODY TO HEPATITIS B SURFACE ANTIGENT (RIAUSURE II-Electro-Nucleonics Laboratories, Inc.)

TEST PROCEDURE

A. Regular Assay

Three positive and seven negative controls should be included in each run regardless of the number of samples to be tested. The control must be tested at the same time as the unknown samples using identical reagents and procedures.

1. Place the cards with reaction tubes on the MAT. Carefully remove the strip cap. It is best removed by upward pressure at the sides of the tubes. Do not pull the strip from an end as this method tends to expel tablets from the tubes.

2. To each of the required and appropriately identified reaction tubes add 0.2 ml of either patient specimen (serum or plasma), Negative Control or Positive Control using a precision pipette. Caution: A new disposable tip must be used for each sample.

3. On the Controller, push the switch marked SAMPLE. The switch lamp will light and a series of agitation/setting cycles will begin. At the end of 24 such cycles (one hour) the sample incubation time is complete. At this time, the lamp in the SAMPLE switch will be extinguished. The Light Emitting Diode (LED) counter will display the number 24. At this stage the test samples may be allowed to stand up to 18 hours before proceeding with rinsing and addition of labelled antibody without significant alteration of results.

4. Add 1.0 ml Buffer Solution to each of the tubes.

5. On the Controller, push the switch marked WASH. The switch lamp will light up and a single cycle of 60 seconds Agitation and 90 seconds Settle will begin. When the cycle is complete, the lamp in the switch will be extinguished and the LED counter will show (1).

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD No. 1410
ANALYTICAL METHOD	RIAUSURE II ANTIBODY TO HEPATITIS B SURFACE ANTIGEN (RIAUSURE II-Electro-Nucleonics Laboratories, Inc.)

TEST PROCEDURE

A. Regular Assay (Continued)

6. Aspirate the contents of each tube allowing for 3-4 mm above the settled CPG glass.

   NOTE: Care should be exercised when aspirating fluid from the reaction tube. Excessive vacuum will cause stirring of the settled CPG and aspiration of particles. Aspiration of CPG will give spurious low CPM. With the 10-place aspirator only a few inches of vacuum is required.

7. Carefully add 0.1 ml of the ^{125}I labeled antibody to each tube. Direct the flow to the bottom of the tube. Do not allow the tip of the delivery pipette to touch the upper part of the tube.

8. On the Controller, push the switch marked LABEL. The switch lamp will light and a series of agitation/settling cycles will begin. At the end of 24 such cycles (one hour), as indicated on the LED counter, the incubation is complete and the lamp in the LABEL switch will be extinguished.

9. Aspirate the contents of the tube allowing for 3-4 mm of liquid above the settled CPG glass particles.

10. Add 1.0 ml Buffer Solution to each tube.

11. On the Controller, push the switch marked WASH. The switch lamp will light and a single cycle of 60 seconds Agitation and 90 seconds Settle will begin. When the cycle is complete, the lamp in the switch will be extinguished and the LED counter will display a (1).

12. Aspirate the Buffer Solution from each tube.

13. Repeat steps 10, 11 and 12 three more times for a total of four washes as shown on the LED counter.

14. Remove the reaction tubes from the cards and place in counting tubes.

15. Count the radioactivity in each reaction tube for 300 seconds with a suitable well-type gamma scintillation counter.

AP000706

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD No. 1410
ANALYTICAL METHOD	RIAUSURE II ANTIBODY TO HEPATITIS B SURFACE ANTIGEN (RIAUSURE II-Electro-Nucleonics Laboratories, Inc.)

TEST PROCEDURE (Continued)

B. Short Assay - See kit booklet

INTERPRETATION OF RESULTS

Presented below is an example and an explanation of the calculations required to determine if unknown samples are reactive for HB_sAg.

Sample No.	Sample Description	CPM
1	Negative Control 1	130
2	Negative Control 2	157
3	Negative Control 3	119
4	Negative Control 4	135
5	Negative Control 5	128
6	Negative Control 6	106
7	Negative Control 7	135
8	Positive Control 1	2,501
9	Positive Control 2	2,431
10	Positive Control 3	2,487
11	Unknown Sample 1	11,556
12	Unknown Sample 2	157
13	Unknown Sample 3	130

1. Determination of the mean of negative controls is obtained by dividing the sum of the net CPM of the negative controls by the number of negative controls.

Negative Control	CPM
1	130
2	157
3	119
4	135
5	128
6	106
7	135
TOTAL	910

$$\frac{\text{Total CPM of negative controls}}{\text{No. negative controls}} = \frac{910}{7} = 130 \text{ CPM}$$

AP000707

5600

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD No. 1410
ANALYTICAL METHOD	RIAUSURE II ANTIBODY TO HEPATITIS B SURFACE ANTIGEN (RIAUSURE II-Electro-Nucleonics Laboratories, Inc.)

INTERPRETATION OF RESULTS (Continued)

2. Those individual negative control values falling outside the range 0.5 to 1.5 times the negative control mean should be discarded.

0.5 x 130 = 65
1.5 x 130 = 195
Acceptable Range - 65 CPM to 195 CPM

In the above, no negative is rejected as aberrant.

3. The negative control need not be revised. Normally, all negative control values should fall within the range of 0.5 and 1.5 times the control mean. If more than one aberrant negative control value is consistently detected, the technique must be suspect and the problem investigated.

4. The net CPM of the mean of the positive control should be at least 5 times the mean of the corrected negative control mean or technique must be suspect and the run repeated.

Positive Control	CPM
1	2,501
2	2,431
3	2,487
TOTAL	7,419

$$\frac{\text{Total CPM of positive controls}}{\text{No. positive controls}} = \frac{7,419}{3} = 2,473 \text{ CPM}$$

$$\frac{\text{Average CPM of positive control}}{\text{Average CPM of negative control}} = \frac{2,473}{130} = 19.0$$

This ratio indicates that the technique is acceptable and the data valid.

5. Calculation of the cut off value:

A. Multiply the net CPM of the negative control mean by 2.0. For Example: 130 x 2.0 = 260 CPM

AP000708

Armour Pharmaceutical Company QUALITY STANDARDS Kankakee, Illinois	APC METHOD No. 1410
ANALYTICAL METHOD	RIAUSURE II ANTIBODY TO HEPATITIS B SURFACE ANTIGEN (RIAUSURE II-Electro-Nucleonics Laboratories, Inc.)

INTERPRETATIONS OF RESULTS (Continued)

5. B. The calculated cut off for positive reactivity in this example is 260 CPM. Therefore, all specimens in this series exceeding 260 net CPM must be considered reactive for HB_sAg.

For Example:

Unknown Sample 1	11,556 CPM
Unknown Sample 2	157 CPM
Unknown Sample 3	130 CPM

NOTE: Many gamma counters do not have the capability for automatically subtracting the background CPM. An alternative to manual subtraction of the background from each sample is to recalculate the negative control as illustrated below:

Gross CPM of the Negative Control Mean = 160 CPM
Instrument Background = 30 CPM

(160 - 30) x 2.0 + 30 = 290 CPM

Therefore, specimens exceeding 290 gross CPM are considered reactive for HB_sAg.

In the examples cited, sample #1 with 11,556 CPM exceeds the cut off value of 290 CPM and is therefore considered reactive for HB_sAg. Samples #2 (157 CPM) and #3 (130 CPM) do not exceed the cut off value and are therefore considered non-reactive for HB_sAg.

NOTES: The analyst is referred to the booklet accompanying the RIAUSURE II Test Kit for Limitations of the TEST PROCEDURE, Specific Performance Characteristics and the complete lists of References.

REFERENCE

Electro-Nucleonics Laboratories, Inc. RIAUSURE II Test Kit Booklet.

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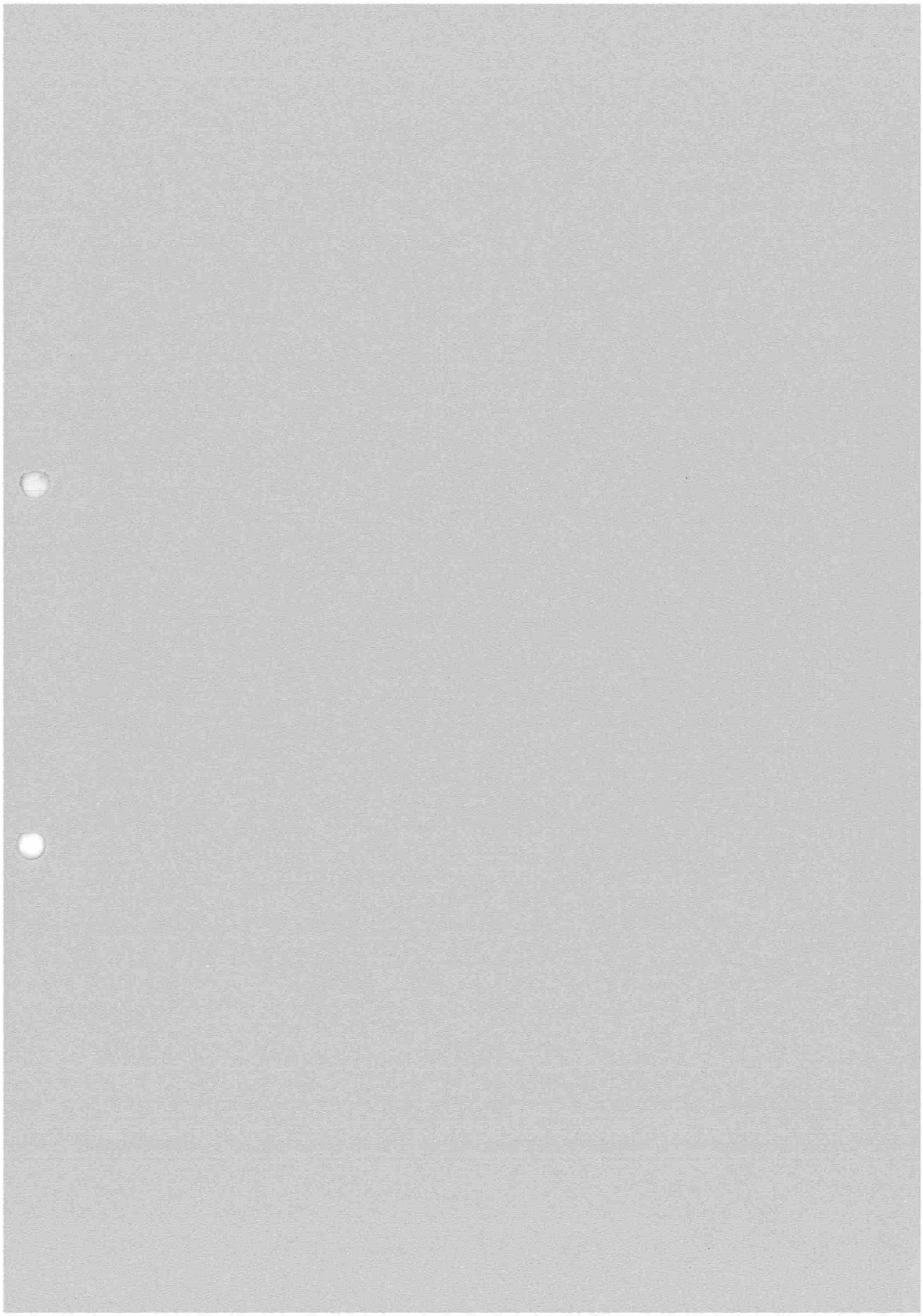
dw

AP000709



APPLICATION FOR A PRODUCT AUTHORISATION FOR

H I G H P O T E N C Y F A C T O R A T E

PART III

PRECLINICAL/EXPERIMENTAL STUDIES

High Potency Factorate is an Anti-haemophilic Fraction used in the treatment of classical haemophilia A or hereditory disease manifested by deficiency of circulating Factor VIII. Factorate products are rich in this Factor which has been isolated from human plasma and the rationale for treatment is the replacement of this blood clotting factor. Consequently Preclinical/Experimental studies in respect of pharmacodynamics, pharmacokinetics and toxicity have not been presented here because:

(a) The material is a protein of human origin and toxicological studies in animal species would be inappropriate.

(b) High Potency Factorate is administered by intravenous injection/ infusion to the site of action which is the circulatory system and pharacokinetic parameters are thus of reduced importance.

(c) The product is identical to the endogenous circulating Factor VIII and is distributed and eliminated by the same pathways.

Each batch of product is tested for presence of foreign (non-human) proteins and for abnormal toxicity.

PART IV

HUMAN PHARMACODYNAMIC AND PHARMACOKINETIC STUDIES

The pharmacokinetic profile of High Potency Factorate (batch K852020) after intravenous injection in human patients has been evaluated by Dr. P. H. Levine, Professor of Medicine at University of Massachusetts Medical School. A copy of his letter to the Company with graphical representations of his results are presented overleaf. The patient records associated with this investigation are incorporated in the Clinical Section of this documentation as part of Dr. Levine's clinical trial. In this study the levels of product in the circulation are represented by increase in the levels of circulating Factor VIII measured by clinical assay.

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The Memorial Hospital
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February 1, 1978

Dr. Carroll O. Johnson
Senior Clinical Research Associate
Armour Pharmaceutical Company
Greyhound Tower
Phoenix, Arizona 85077

Dear Dr. Johnson:

Enclosed are six sets of data with regard to our trial of your new Factor VIII preparations AL-1259 (lot number 852030). We are returning any remaining unused material to you under separate cover.

As you will note from the enclosed data, we found your AHF to produce excellent yields as determined by in vivo assay. After the initial rapid fall-off seen after injection of all Factor VIII preparations, your material exhibits a half-life in the circulation in the range of 8 - 12 hours. It would appear to be better than average for a product of this type.

Sincerely yours,

GRO-C

Peter H. Levine, M.D.
Chief, Medical Division
Professor of Medicine, U. Mass. Medical School
Director, New England Area Comprehensive
Hemophilia Center

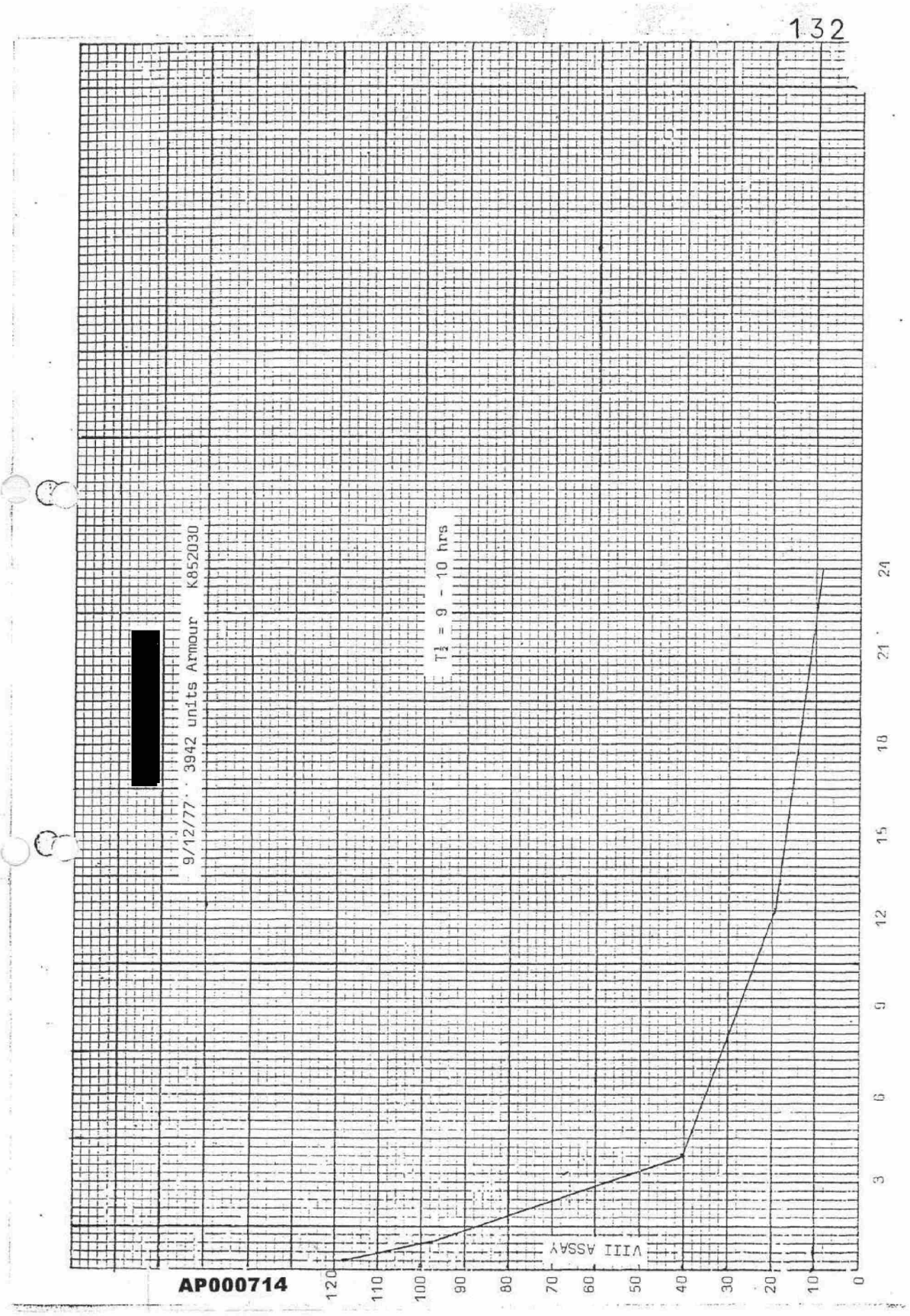
PHL:cem

Enclosures

AP000713

ARMOUR000921

ARMO0000092_0135



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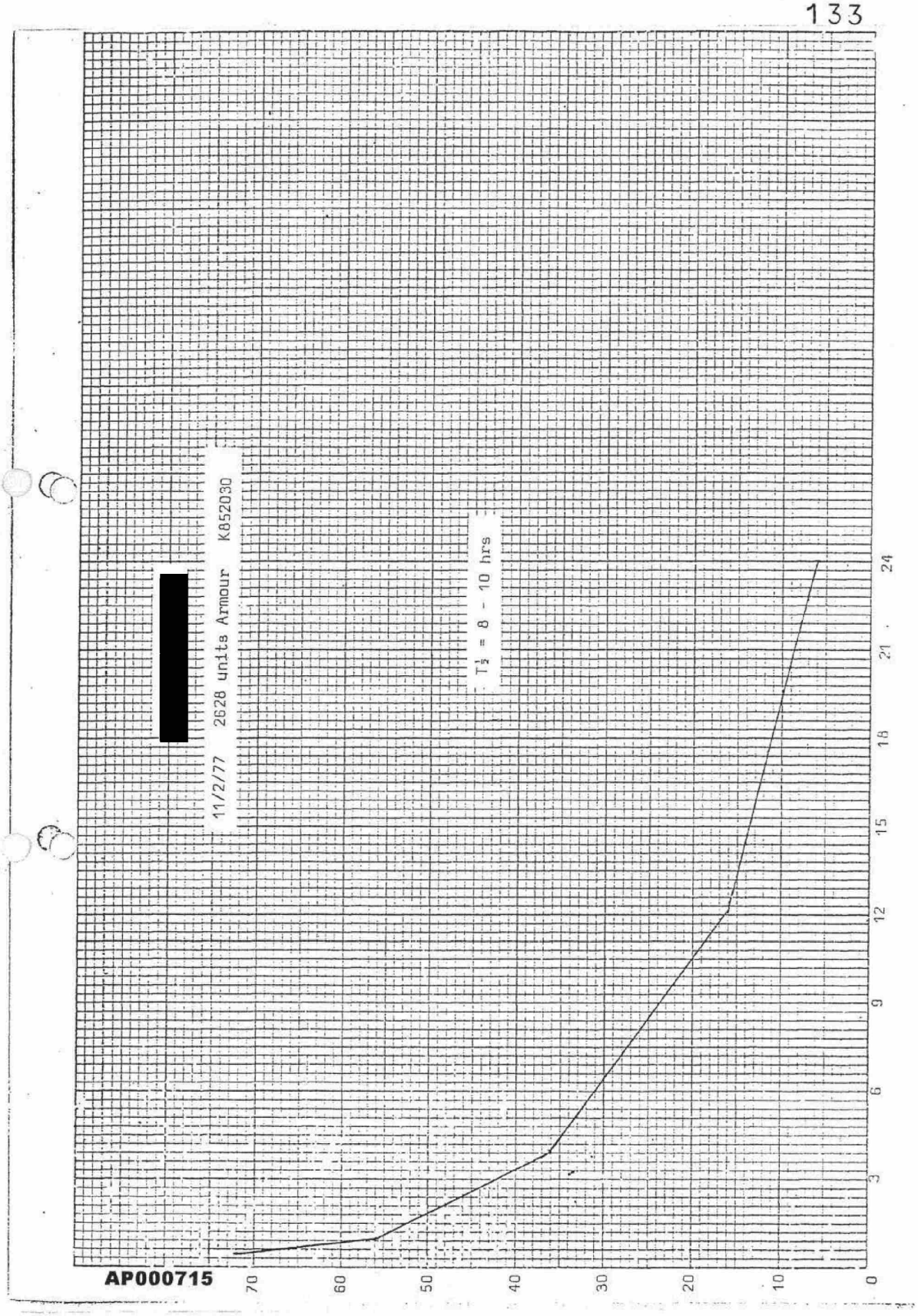

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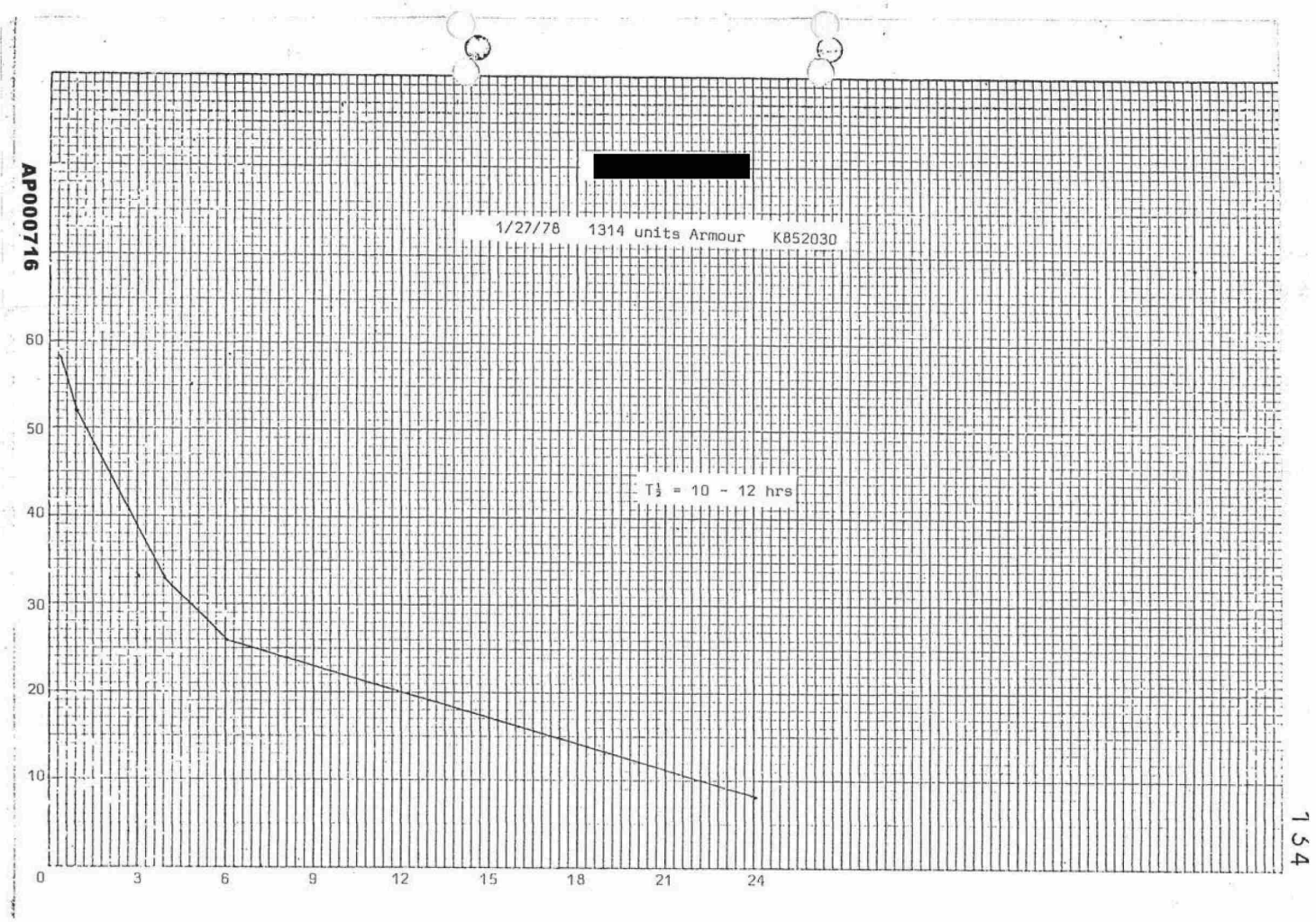

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ARMOUR000924

PART V CLINICAL TRIALS

1.1. Summary of Clinical Evaluation of AL-1259 - High Potency Factorate - Protocol 101

1.1.1. Introduction

Human blood contains factors which interact to produce blood coagulation. Haemorrhagic disorders occur when one or more factors are absent or decreased. Factor VIII is a plasma protein whose congenital deficiency results in the bleeding disorder known as Haemophilia A.

Factor VIII deficiencies may be corrected with fresh normal plasma, crude concentrates such as cryoprecipitate, anti-haemophilic globulin (Cohn Fraction I), or highly purified and concentrated preparations of Factor VIII. Factor VIII concentrates are required to avert over-loading of the circulatory system when a large amount of Factor VIII is required for control of haemorrhage as in surgery or trauma. With clinical usage in these situations requiring Factor VIII in unit amounts of thousands or tens of thousands, a more concentrated and highly purified form of Factor VIII is required. A change in method of manufacture has enabled AL-1259 to meet these criteria. This product will have more AHF units per volume and have less protein. It is a concentrate of Factor VIII made by a modification of the Cohn Fractionation process, yielding not less than 30 units of AHF per ml. The AHF specific activity is approximately 1.0 AHF unit/mg total protein. The solution time will be not more than 30 minutes and will generally reconstitute within 10 minutes. Isoagglutinin titres will be assayed for each lot. AL-1259 is produced from Source Plasma (Human) that has been tested and found negative for HbsAg using a Third Generation test. The final product is similarly tested and found negative for HbsAg. Heparin content is not more than 30 units/30 ml of reconstituted vial.

1.1.2. Objective

This study is designed to demonstrate the safety and efficacy of AL-1259 in treating those patients requiring large amounts of Factor VIII as preparation of elective surgery or to control severe haemorrhage resulting from surgery or trauma.

1.1.3. Materials and Methods

(a) Selection of Patients

i) Criteria for Inclusion

Patients previously diagnosed as deficient in Antihaemophilic Factor (AHF - Factor VIII) and in need of large prophylactic and therapeutic replacement of Factor VIII are suitable candidates for this study. The subjects will be presented with the objectives of the study, have the possible hazards explained and, if willing to participate in the study, sign informed consent forms.

ii) Criteria for Exclusion

Subjects with known inhibitors of Factor VIII

Any other disease likely to interfere with evaluation of or to prevent completion of the study.

iii) Concurrent Medication

Administration of other types of Antihaemophilic Factor during the course of this study is to be avoided, if possible.

All concurrent medications used during the study will be listed on the case report forms.

(b) Supplies and Dosage

i) AL-1259 represents an AHF concentrate in the high range of potency. It is intended only for intravenous infusion and will be supplied in 50 ml vials of not less than 900 units of AHF to be reconstituted with 30 ml of sterile water for injection. AL-1259 and diluent are to be stored at refrigerator temperature ($2^{o}C$ - $8^{o}C$). Freezing may damage the container for the diluent.

ii) The determination of dosage will be based on the amount of AL-1259 required to reach desired levels of AHF based on the weight of the patient as calculated by the formula:

$$\frac{\text{Weight in Pounds}}{4.4} \times \text{AHF Activity desired} =$$

Number of units of AHF needed

1.1.4. Procedures and Evaluation

1.1.4.1. Plan of Study

i) A brief medical history and physical examination will be performed shortly before and at the conclusion of the study. Appropriate information will be recorded on the case report form.

ii) Laboratory Studies

a. Laboratory studies to be performed include:

Factor VIII (%)
Pre-infusion the post-infusion

Factor VIII (Half-Life) - on select cases only
Single dose infusion

Haemoglobin
Pre-infusion

Haematocrit
Pre-infusion

HbsAg - Antigen, Antibody
Pre-infusion

Bilirubin
Pre-infusion

S.G.O.T.
Pre-infusion

iii) Observations During Infusion

a. Following each intravenous infusion of AL-1259 careful observation will be made with notation of any local or systemic reactions. All symptoms will be recorded and evaluated for relationship to the injection. A reaction which, in the judgment of the investigator, endangers the volunteer or shows failure of appropriate response to AL-1259 will be grounds for discontinuing further injections in that volunteer. Appropriate therapeutic measures and follow-up will be instituted. Reactions severe enough to cause discontinuation of the study should be reported to Armour Pharmaceutical Company as soon as possible.

1.1.4.2. Evaluation of AL-1259 will be based on the following:

i) Ability to promote effective haemostasis, when applicable. It is well known that an achieved increase in Factor VIII gives haemostasis.

ii) Ability to reach calculated levels of Factor VIII.

iii) Ability to maintain predicted levels (half-life) of Factor VIII.

iv) Incidence and severity of adverse reactions.

1.1.4.3. Recording of Results

i) The sponsor will provide report forms on which all laboratory results and clinical evaluations will be recorded for each volunteer.

ii) As each subject completes the study, the investigator will promptly submit the typewritten case report to:

Karl B. Hansen M.D.,
Armour Pharmaceutical Company,
Greyhound Tower,
Phoenix, Arizona 85077.

iii) Federal law requires that following completion of a clinical study a copy of all records of that study is maintained by the Clinical Investigator for a minimum of two years. If requested, the sponsor will, upon receipt of individual case report forms, provide each investigator with a copy of such records for his files.

1.1.5. Unused Medication

1.1.5.1. In accordance with federal law, all unused medications must be accounted for and returned to the sponsor at the conclusion of the study.

Clinical evaluation of three lots of AL-1259 for safety, potency and efficacy was carried out by the following investigators at four institutions:

STUDY 1 - Harold R. Roberts, M.D.
and
Philip M. Blatt, M.D.,
North Carolina Memorial Hospital,
University of North Carolina,
Chapel Hill, North Carolina 27514
Lot No. K852031

STUDY 2 - Louis M. Aledort, M.D.,
Mount Sinai School of Medicine,
New York, New York 10029,
Lot No. K852032

STUDY 3 - Margaret W. Hilgartner, M.D.,
The New York Hospital,
Cornell Medical Center,
New York, New York 10021,
Lot No. K852032

STUDY 4 - Peter H. Levine, M.D.,
The Memorial Hospital,
Worcester, Massachusetts 01605,
Lot No. K852030

Each investigator accepted Protocol No. 101 as the guide for his study and completed a case report for each patient.

1.2. Summary of Trials Reported

The following information summarises the four separate evaluations of High Potency Factorate (AL-1259):-

1.2.1. Number of Trials

Four.

1.2.2. Number of Patients Entering Trials

Twenty-eight.

1.2.3. Number Receiving Test Medication and Number Withdrawn

Twenty-eight (none withdrawn).

1.2.4. Daily Dosage Expressed as Mean

Infusion from 1,218 to 5,000 AHF units.

1.2.5. Duration of Dosage

Single infusions.

1.2.6. Summary Results in Terms of Efficacy and Other Statistics - (see attached Table)

Investigators who have wide experience with blood products made no comments when the levels of Factor VIII were less than expected. The ability to reach calculated levels was noted by three quarters of the investigators. No allergic reactions reported. Previous allergic effects seen with cryoprecipitate in 6 patients (Dr. Roberts) and 1 patient (Dr. Aledort).

1.2.7. Adverse Reactions

In one case only was an adverse reaction reported. In this patient the following events took place and was thought by the clinician to be a case of 'short incubation' (non-A, non-B) hepatitis.

2nd August, 1977 - Infusion of AL-1259
12th August, 1977 - Malaise and chills
14th August, 1977 - Dark urine
16th August, 1977 - Nausea and vomiting, icterus and the following clinical parameters:-

Total Bilirubin	6.0
SGOT	1855
SGPT	2002
HBsAg	Negative

14th September, 1977 - Clinically entirely well

Total Bilirubin	6.0
SGOT	42
SGPT	47

1.2.8. Conclusions

In general, all investigators found the product to be safe, potent and efficacious and no adverse reactions were observed except for the one possibly related case of hepatitis.

It is of especial interest that no allergic reactions occurred in view of the history of allergic reactions to cryoprecipitate in 7/28 patients.

The levels of Factor VIII achieved were higher than calculated in all studies with the exception of Study 3 (Dr. Hilgartner).

OVERALL RESULTS OF STUDY

Evaluation By	No. of Patients Entering	No. Receiving Test Medication	No. Withdrawn	Dosage (Range)	Adverse Effects	Efficacy And Other Statistics	Batch Used
Dr. H.R. Roberts and Dr. P.M. Blatt	10	10	None	2250 - 5000 AHF units	1 case (RB) with Hepatitis 10 days after infusion	The one adverse effect cannot necessarily be attributed to AL-1259. AHF values obtained *in vivo* were 10 - 50% more than expected.	K852031
Dr. L.M. Aledort	8	8	None	1218 - 4872 AHF units	None	AHF values obtained *in vivo* approximately 30% more than expected values	K852032
Dr. M.W. Hilgartner	5 (3 children) (2 adults)	5	None	1218 - 2436 AHF units	None	AHF values obtained *in vivo* were 83% of expected values. (With exception of patient MC)	K852032
Dr. P.H. Levine	5 (1 child) (4 adults)	5	None	1214 - 3942 AHF units	None	AHF values obtained *in vivo* were approximately 16% more than expected values.	K852030

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1.3. STUDY 1 - Evaulation of AL-1259 by Drs. H. R. Roberts and P. M. Blatt

Drs. Roberts and Blatt studied ten adult patients with the established diagnosis of Haemophilia A. One patient was treated for a joint haemorrhage and the other nine received prophylactic treatment. Recovery of AHF activity in-vivo averaged 133% of the calculated value. One patient (RB) developed hepatitis (HBsAg negative) ten days after the infusion of AL-1259. He made a prompt and complete recovery and showed no evidence of liver damage on follow-up one month later. In view of the facts that this patient had received 30 bags of cryoprecipitate during the previous three months and that his hepatitis followed the infusion of AL-1259 after an interval shorter than the usually accepted inclubation period for post-transfusion hepatitis, this case of hepatitis is not necessarily to be attributed to AL-1259 but is reported for information. No other adverse effects were observed in any of the ten patients. Results are tabulated overleaf.

Clinical Abstract Patient [redacted]

Six bags of cryoprecipitate on each of the following dates: 29th May, 1977, 32st May 1977, 1st June, 1977, 26th June, 1977 and 5th July, 1977.

On 2nd August, 1977	- infusion of AL-1259
On 12th August, 1977	- malaise and chills
On 14th August, 1977	- dark urine
On 16th August, 1977	- nausea, vomiting, icterus, following laboratory values: Bilirubin total 6.0, direct 2.9, SGOT 1855, SGPT 2002 HBsAg negative
On 14th September, 1977	- Clinically entirely well, laboratory values: bilirubin total 6.0, direct 0.2, SGOT 42, SGPT 47.

STUDY 1 - HAROLD R. ROBERTS. M.D. AND PHILIP M. BLATT, M.D.

TABULATED RESULTS

Patient	Age	Wt. Kg.	Treatment	Dose* Units	Calculated Rise % AHF	Actual Rise % AHF	Adverse Effects
[redacted]	19	57	Joint Haemorrhage	2438	98	128	None
[redacted]	29	54.4	Prophylactic	2250	94	132	None
[redacted]	32	78.3	Prophylactic	3187	90	130	None
[redacted]	38	100	Prophylactic	5000	100	134	None
[redacted]	37	90	Prophylactic	4500	100	130	None
[redacted]	32	68	Prophylactic	3750	125	173	None
[redacted]	25	95	Prophylactic	4400	105	142	None
[redacted]	21	55	Prophylactic	2250	100	150	Hepatitis
[redacted]	28	83	Prophylactic	3957	107	121	None
[redacted]	21	82	Prophylactic	4375	105	117	None

*AL-1259 Lot K852031, 1164 AHF units per vial

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ARMOUR000933

ARMO0000092_0147

ANTIHEMOPHILIC FACTOR (HUMAN)
Protocol No. 101

144

Patient Identification [redacted] Study No. 1

Age 19 *(yrs)* Weight ____ *(kg)* or 125 *(lbs)* Height 71 *(in)* Case No. 1

MEDICAL HISTORY

Kidney Disease	[X] No	[ ] Yes	Hepatitis	[ ] No	[X] Yes	Date / ?/
Hypertension	[X] No	[ ] Yes	Jaundice	[X] No	[ ] Yes	Date / /
Uremia	[X] No	[ ] Yes	HAA Test	[X] Negative	[ ] Positive	Date 10/10/76

REACTIONS FROM *PREVIOUS* THERAPY?

[ ] No [X] Yes; *please specify therapy and reaction:*

Itching, hives, etc., associated with cryoprecipitate

PHYSICAL EXAMINATION

Number and list each positive finding in pre-treatment examination. On post-treatment examination, use same number and indicate any changes in each finding. Use additional numbers to list any new positive findings.

Pre-Treatment	Post-Treatment
L. big toe, left knee, left hip	

DIAGNOSIS

[ ] Prophylaxis [X] Joint Hemorrhage [ ] Muscle Hemorrhage

[ ] Overt Bleeding [ ] Massive Wound [ ] Surgery

CONCOMITANT MEDICATIONS
(In use at enrollment or begun during study)

DRUG	SINGLE DOSE	ROUTE *(IM, IV, PO)*	SCHEDULE	DATE STARTED	DATE STOPPED

AP000726

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ARMO0000092_0148

Date	Hemoglobin	Hematocrit	HAA	Bilirubin	S.G.O.T.	
8/3/77	12.9	39.0	Neg.	.9	24	

AL-1259 ADMINISTRATION

Lot No. K852031 Dosage 2437.5 AHF units in 65 ml

AHF units/vial 1164 Solution Time 90 *(minutes)* Infusion Time 15 *(minutes)*

Calculated AHF Rise 98 % Actual AHF Rise 128 %

	Date	Time	B.P.	Pulse	Temp.	PTT	AHF%
Preinfusion	8/3		118/78	65			<.01 u/ml
15 minutes Immediate Post Infusion	"						1.28
1 hr " "	"						1.07
3 hr " "	"						.84
6 hr " "	"						.79
9 hr " "	"						.64
12 hr " "	"						.43

ADVERSE EFFECT

[X] No adverse effects [ ] Adverse effects as in table:

SYMPTOMS OF SIGNS	DATE AND TIME OF ONSET	DURATION	SEVERITY*

*Classify reaction as 1 = mild; 2 = moderate; 3 = severe

Clinical Response Chronic hemophilic arthropathy. Post treatment without change (the same).

AP000727

_____, M.D. Date 9/13/77

Signature of Investigator

ANTIHEMOPHILIC FACTOR (HUMAN)
Protocol No. 101

146

Patient Identification [redacted] Study No. 1

Age 29 *(yrs)* Weight 54.4 *(kg)* or 120 *(lbs)* Height 64 *(in)* Case No. 2

MEDICAL HISTORY

Kidney Disease	[X] No	[ ] Yes	Hepatitis	[X] No	[ ] Yes	Date	
Hypertension	[X] No	[ ] Yes	Jaundice	[X] No	[ ] Yes	Date	
Uremia	[X] No	[ ] Yes	HAA Test	[X] Negative	[ ] Positive	Date	1/10/77

REACTIONS FROM *PREVIOUS* THERAPY?

[X] No [ ] Yes; *please specify therapy and reaction:*

PHYSICAL EXAMINATION

Number and list each positive finding in pre-treatment examination. On post-treatment examination, use same number and indicate any changes in each finding. Use additional numbers to list any new positive findings.

Pre-Treatment	Post-Treatment

DIAGNOSIS

[X] Prophylaxis [ ] Joint Hemorrhage [ ] Muscle Hemorrhage

[ ] Overt Bleeding [ ] Massive Wound [ ] Surgery

CONCOMITANT MEDICATIONS
(In use at enrollment or begun during study)

DRUG	SINGLE DOSE	ROUTE *(IM, IV, PO)*	SCHEDULE	DATE STARTED	DATE STOPPED
			AP000728		

ARMOUR000936

Date	Hemoglobin	Hematocrit	HAA	Bilirubin	S.G.O.T.	147
8/1/77	14.2 (1/77)	43.0 (1/77)			Normal	

AL-1259 ADMINISTRATION

Lot No. K852031 Dosage 2250 AHF units in 60 ml

AHF units/vial 1184 Solution Time 120 *(minutes)* Infusion Time 15 *(minutes)*

Calculated AHF Rise 94 % Actual AHF Rise 132 %

	Date	Time	B.P.	Pulse	Temp.	PTT	AHF%
Preinfusion	8/1	8:30	110/70	82			.01
15 minutes Immediate Post Infusion		8:45					1.32
1 hr " "		9:45					1.03
3 hr " "		11:45					.90
6 hr " "		2:45					.80
9 hr " "		5:45					.69
12 hr " "		8:45					.35

ADVERSE EFFECT

[x] No adverse effects [ ] Adverse effects as in table:

SYMPTOMS OF SIGNS	DATE AND TIME OF ONSET	DURATION	SEVERITY*

*Classify reaction as1 = mild; 2 = moderate; 3 = severe

Clinical Response The patient was not bleeding prior to infusion, therefore, clinical response could not be evaluated.

AP000729

_____, M.D. Date 9/13/77

Signature of Investigator

ANTIHEMOPHILIC FACTOR (HUMAN)
Protocol No. 101

148

Patient Identification [redacted] Study No. 1

Age 32 (yrs) Weight 78.3 (kg) or 177 (lbs) Height 75 (in) Case No. 3

MEDICAL HISTORY

Kidney Disease	[X] No	[ ] Yes	Hepatitis	[X] No	[ ] Yes	Date	
Hypertension	[X] No	[ ] Yes	Jaundice	[X] No	[ ] Yes	Date	
Uremia	[X] No	[ ] Yes	HAA Test	[X] Negative	[ ] Positive	Date	8/4/77

REACTIONS FROM *PREVIOUS* THERAPY?

[ ] No [X] Yes; *please specify therapy and reaction:*

Itching and hives with cryoprecipitate and fresh frozen plasma.

PHYSICAL EXAMINATION

Number and list each positive finding in pre-treatment examination. On post-treatment examination, use same number and indicate any changes in each finding. Use additional numbers to list any new positive findings.

Pre-Treatment	Post-Treatment

DIAGNOSIS

[X] Prophylaxis [ ] Joint Hemorrhage [ ] Muscle Hemorrhage

[ ] Overt Bleeding [ ] Massive Wound [ ] Surgery

CONCOMITANT MEDICATIONS
(In use at enrollment or begun during study)

DRUG	SINGLE DOSE	ROUTE (IM, IV, PO)	SCHEDULE	DATE STARTED	DATE STOPPED
None					

AP000730

ARMOUR000938

Date	Hemoglobin	Hematocrit	HAA	Bilirubin	S.G.O.T.	
8/1/77	14.2 (12-20-76)	43.0 (12-20-76)	Neg.		76	

AL-1259 ADMINISTRATION

Lot No. K852031 Dosage 3187 AHF units in 85 ml

AHF units/vial 1164 Solution Time 120 *(minutes)* Infusion Time 15 *(minutes)*

Calculated AHF Rise 90 % Actual AHF Rise 130 %

	Date	Time	B.P.	Pulse	Temp.	PTT	AHF%
Preinfusion	8/1		126/80	80			.01
15 minutes Immediate Post Infusion							1.30
1 hr " "							1.06
3 hr " "							.94
6 hr " "							.85
9 hr " "							.67
12 hr " "							.30

ADVERSE EFFECT

[X] No adverse effects [ ] Adverse effects as in table:

SYMPTOMS OF SIGNS	DATE AND TIME OF ONSET	DURATION	SEVERITY*

*Classify reaction as 1 = mild; 2 = moderate; 3 = severe

Clinical Response Patient not bleeding prior to infusion, therefore, clinical response could not be evaluated.

AP000731

_____, M.D. Date 9/13/77

Signature of Investigator

ANTIHEMOPHILIC FACTOR (HUMAN)
Protocol No. 101

150

Patient Identification [redacted] Study No. 1

Age 38 *(yrs)* Weight 100 *(kg)* or 217 *(lbs)* Height 72 *(in)* Case No. 4

MEDICAL HISTORY

Kidney Disease	[X] No	[ ] Yes	Hepatitis	[X] No	[ ] Yes	Date
Hypertension	[X] No	[ ] Yes	Jaundice	[X] No	[ ] Yes	Date
Uremia	[X] No	[ ] Yes	HAA Test	[X] Negative	[ ] Positive	Date 8/4/77

REACTIONS FROM *PREVIOUS* THERAPY?

[ ] No [X] Yes; *please specify therapy and reaction:*

Itching associated with whole blood transfusion

PHYSICAL EXAMINATION

Number and list each positive finding in pre-treatment examination. On post-treatment examination, use same number and indicate any changes in each finding. Use additional numbers to list any new positive findings.

Pre-Treatment	Post-Treatment

DIAGNOSIS

[X] Prophylaxis [ ] Joint Hemorrhage [ ] Muscle Hemorrhage

[ ] Overt Bleeding [ ] Massive Wound [ ] Surgery

CONCOMITANT MEDICATIONS
(In use at enrollment or begun during study)

DRUG	SINGLE DOSE	ROUTE *(IM, IV, PO)*	SCHEDULE	DATE STARTED	DATE STOPPED
None					
			AP000732		

Date	Hemoglobin	Hematocrit	HAA	Bilirubin	S.G.O.T.	151
9/4/77	15.6	45.0	Neg.	.5	27	

AL-1259 ADMINISTRATION

Lot No. K85031 Dosage 5000 AHF units in 134 ml

AHF units/vial 1164 Solution Time 40 *(minutes)* Infusion Time 15 *(minutes)*

Calculated AHF Rise 100 % Actual AHF Rise 134 %

	Date	Time	B.P.	Pulse	Temp.	PTT	AHF%
Preinfusion	8/4		130/90	76			.01
15 minutes Immediate Post Infusion							1.34
1 hr " "							1.06
3 hr " "							.93
6 hr " "							.87
9 hr " "							.70
12 hr " "							.34

ADVERSE EFFECT

[X] No adverse effects [ ] Adverse effects as in table:

SYMPTOMS OF SIGNS	DATE AND TIME OF ONSET	DURATION	SEVERITY*

*Classify reaction as1 = mild; 2 = moderate; 3 = severe

Clinical Response The patient was not bleeding prior to infusion, therefore clinical response could not be evaluated.

AP000733

, M.D. Date 9/13/77

Signature of Investigator

ANTIHEMOPHILIC FACTOR (HUMAN)
Protocol No. 101

152

Patient Identification [redacted] Study No. 1

Age 37 (yrs) Weight 90 (kg) or 194 (lbs) Height 68 (in) Case No. 5

MEDICAL HISTORY

Kidney Disease	[X] No	[ ] Yes	Hepatitis	[ ] No	[X] Yes	Date 1/5/77
Hypertension	[X] No	[ ] Yes	Jaundice	[X] No	[ ] Yes	Date
Uremia	[X] No	[ ] Yes	HAA Test	[X] Negative	[ ] Positive	Date 8/4/77

REACTIONS FROM *PREVIOUS* THERAPY?

[X] No [ ] Yes; *please specify therapy and reaction:*

PHYSICAL EXAMINATION

Number and list each positive finding in pre-treatment examination. On post-treatment examination, use same number and indicate any changes in each finding. Use additional numbers to list any new positive findings.

Pre-Treatment	Post-Treatment

DIAGNOSIS

[X] Prophylaxis [ ] Joint Hemorrhage [ ] Muscle Hemorrhage

[ ] Overt Bleeding [ ] Massive Wound [ ] Surgery

CONCOMITANT MEDICATIONS
(In use at enrollment or begun during study)

DRUG	SINGLE DOSE	ROUTE (IM, IV, PO)	SCHEDULE	DATE STARTED	DATE STOPPED
None					
			AP000734		

ARMOUR000942

Date	Hemoglobin	Hematocrit	HAA	Bilirubin	S.G.O.T.	153
8/4/77	16.7	50.0	Neg.	.5	79.0	

AL-1259 ADMINISTRATION

Lot No. K852031 Dosage 4500 AHF units in 110 ml

AHF units/vial 1164 Solution Time 60 *(minutes)* Infusion Time 15 *(minutes)*

Calculated AHF Rise 100 % Actual AHF Rise 130 %

	Date	Time	B.P.	Pulse	Temp.	PTT	AHF%
Preinfusion	8/4		145/80	78			.01
15 minutes Immediate Post Infusion							1.30
1 hr " "							1.03
3 hr " "							.90
6 hr " "							.83
9 hr " "							.71
12 hr " "							.37

ADVERSE EFFECT

[x] No adverse effects [ ] Adverse effects as in table:

SYMPTOMS OF SIGNS	DATE AND TIME OF ONSET	DURATION	SEVERITY*

*Classify reaction as 1 = mild; 2 = moderate; 3 = severe

Clinical Response The patient was not bleeding prior to infusion therefore clinical response could not be evaluated.

AP000735

_____, M.D. Date 9/13/77

Signature of Investigator

ANTIHEMOPHILIC FACTOR (HUMAN)
Protocol No. 101

154

Patient Identification [redacted] Study No. 1

Age 32 (yrs) Weight ____ (kg) or 150 (lbs) Height 66 (in) Case No. 6

MEDICAL HISTORY

Kidney Disease	[X] No	[ ] Yes	Hepatitis	[X] No	[ ] Yes	Date 8/3/77
Hypertension	[X] No	[ ] Yes	Jaundice	[X] No	[ ] Yes	Date 8/3/77
Uremia	[X] No	[ ] Yes	HAA Test	[X] Negative	[ ] Positive	Date 8/3/77

REACTIONS FROM *PREVIOUS* THERAPY?

[ ] No [X] Yes; *please specify therapy and reaction:*

Itching and hives from cryoprecipitate and plasma

PHYSICAL EXAMINATION

Number and list each positive finding in pre-treatment examination. On post-treatment examination, use same number and indicate any changes in each finding. Use additional numbers to list any new positive findings.

Pre-Treatment	Post-Treatment

DIAGNOSIS

[X] Prophylaxis [ ] Joint Hemorrhage [ ] Muscle Hemorrhage

[ ] Overt Bleeding [ ] Massive Wound [ ] Surgery

CONCOMITANT MEDICATIONS
(In use at enrollment or begun during study)

DRUG	SINGLE DOSE	ROUTE (IM, IV, PO)	SCHEDULE	DATE STARTED	DATE STOPPED
None					
			AP000736		

ARMOUR000944

ARMO0000092_0158

Date	Hemoglobin	Hematocrit	HAA	Bilirubin	S.G.O.T.	155
8/3/77	17.8	54.0	Neg.	.6	66	

AL-1259 ADMINISTRATION

Lot No. K852031 Dosage 3750 AHF units in 100 ml

AHF units/vial 1164 Solution Time 90 *(minutes)* Infusion Time 15 *(minutes)*

Calculated AHF Rise 125 % Actual AHF Rise 173 %

	Date	Time	B.P.	Pulse	Temp.	PTT	AHF%
Preinfusion	8/3		128/80	75			<.01 u/ml
15 minutes Immediate Post Infusion	"						1.73
1 hr " "	"						1.48
3 hr " "	"						1.13
6 hr " "	"						1.01
9 hr " "	"						.95
12 hr " "							.57

ADVERSE EFFECT

[X] No adverse effects [ ] Adverse effects as in table:

SYMPTOMS OF SIGNS	DATE AND TIME OF ONSET	DURATION	SEVERITY*

*Classify reaction as 1 = mild; 2 = moderate; 3 = severe

Clinical Response The patient was not bleeding prior to infusion, therefore, clinical response could not be evaluated.

AP000737

_____, M.D. Date 9/13/77

Signature of Investigator

ARMOUR000945

ARMO0000092_0159

ANTIHEMOPHILIC FACTOR (HUMAN)
Protocol No. 101

156

Patient Identification [redacted] Study No. 1

Age 25 (yrs) Weight ____ (kg) or 208 (lbs) Height 73 (in) Case No. 7

MEDICAL HISTORY

Kidney Disease	[X] No	[ ] Yes	Hepatitis	[X] No	[ ] Yes	Date 8/3/77
Hypertension	[X] No	[ ] Yes	Jaundice	[X] No	[ ] Yes	Date 8/3/77
Uremia	[X] No	[ ] Yes	HAA Test	[X] Negative	[ ] Positive	Date 8/3/77

REACTIONS FROM *PREVIOUS* THERAPY?

[X] No [ ] Yes; *please specify therapy and reaction:*

PHYSICAL EXAMINATION

Number and list each positive finding in pre-treatment examination. On post-treatment examination, use same number and indicate any changes in each finding. Use additional numbers to list any new positive findings.

Pre-Treatment	Post-Treatment

DIAGNOSIS

[X] Prophylaxis [ ] Joint Hemorrhage [ ] Muscle Hemorrhage

[ ] Overt Bleeding [ ] Massive Wound [ ] Surgery

CONCOMITANT MEDICATIONS
(In use at enrollment or begun during study)

DRUG	SINGLE DOSE	ROUTE (IM, IV, PO)	SCHEDULE	DATE STARTED	DATE STOPPED
None					

AP000738

ARMOUR000946

ARMO0000092_0160

Date	Hemoglobin	Hematocrit	HAA	Bilirubin	S.G.O.T.	157
8/3/77	17.5	50.0	Neg.	1.0	144	

AL-1259 ADMINISTRATION

Lot No. K852031 Dosage 4400 AHF units in 120 ml

AHF units/vial 1164 Solution Time 60 *(minutes)* Infusion Time 15 *(minutes)*

Calculated AHF Rise 105 % Actual AHF Rise 142 %

	Date	Time	B.P.	Pulse	Temp.	PTT	AHF%
Preinfusion	8/3		135/85	78			<.01
15 minutes Immediate Post Infusion							1.42
1 hr " "							1.20
3 hr " "							1.03
6 hr " "							.92
9 hr " "							.83
12 hr " "							.51

ADVERSE EFFECT

[X] No adverse effects [ ] Adverse effects as in table:

SYMPTOMS OF SIGNS	DATE AND TIME OF ONSET	DURATION	SEVERITY*

*Classify reaction as 1 = mild; 2 = moderate; 3 = severe

Clinical Response The patient was not bleeding prior to infusion, therefore, clinical response could not be evaluated.

AP000739

_____, M.D. Date 9/13/77

Signature of Investigator

ARMOUR000947

ANTIHEMOPHILIC FACTOR (HUMAN)
Protocol No. 101

158

Patient Identification [redacted] Study No. 1

Age 21 (yrs) Weight ____ (kg) or 118 (lbs) Height 71 (in) Case No. 8

MEDICAL HISTORY

Kidney Disease	[X] No	[ ] Yes	Hepatitis	[X] No	[ ] Yes	Date 8/2/77
Hypertension	[X] No	[ ] Yes	Jaundice	[X] No	[ ] Yes	Date 8/2/77
Uremia	[X] No	[ ] Yes	HAA Test	[X] Negative	[ ] Positive	Date 8/2/77

REACTIONS FROM *PREVIOUS* THERAPY?

[X] No [ ] Yes; *please specify therapy and reaction:*

PHYSICAL EXAMINATION

Number and list each positive finding in pre-treatment examination. On post-treatment examination, use same number and indicate any changes in each finding. Use additional numbers to list any new positive findings.

Pre-Treatment	Post-Treatment

DIAGNOSIS

[X] Prophylaxis [ ] Joint Hemorrhage [ ] Muscle Hemorrhage

[ ] Overt Bleeding [ ] Massive Wound [ ] Surgery

CONCOMITANT MEDICATIONS
(In use at enrollment or begun during study)

DRUG	SINGLE DOSE	ROUTE (IM, IV, PO)	SCHEDULE	DATE STARTED	DATE STOPPED
None					
			AP000740		

ARMOUR000948

Date	Hemoglobin	Hematocrit	HAA	Bilirubin	S.G.O.T.	
8/2/77	15.2	46.0	Neg.		30	

AL-1259 ADMINISTRATION

Lot No. K852031 Dosage 2250 AHF units in 60 ml

AHF units/vial 1164 Solution Time 90 *(minutes)* Infusion Time 15 *(minutes)*

Calculated AHF Rise 100 % Actual AHF Rise 150 %

	Date	Time	B.P.	Pulse	Temp.	PTT	AHF%
Preinfusion	8/2/77		122/80	75			<.01
15 minutes Immediate Post Infusion							1.50
1 hr " "							1.20
3 hr " "							1.01
6 hr " "							.92
9 hr " "							.83
12 hr " "							.45

ADVERSE EFFECT

☐ No adverse effects ☒ Adverse effects as in table:

SYMPTOMS OF SIGNS	DATE AND TIME OF ONSET	DURATION	SEVERITY*
Hepatitis - full report already sent to Mr. Johnson			

*Classify reaction as 1 = mild; 2 = moderate; 3 = severe

Clinical Response The patient was not bleeding prior to infusion, therefore, clinical response could not be evaluated.

AP000741

_____, M.D. Date 9/13/77

Signature of Investigator

ARMOUR000949

ANTIHEMOPHILIC FACTOR (HUMAN)
Protocol No. 101

160

Patient Identification [redacted] Study No. 1

Age 28 (yrs) Weight ____ (kg) or 182 (lbs) Height 67 (in) Case No. 9

MEDICAL HISTORY

Kidney Disease	[X] No	[ ] Yes	Hepatitis	[ ] No	[X] Yes	Date 4//76
Hypertension	[X] No	[ ] Yes	Jaundice	[X] No	[ ] Yes	Date
Uremia	[X] No	[ ] Yes	HAA Test	[X] Negative	[ ] Positive	Date 12/9/76

REACTIONS FROM *PREVIOUS* THERAPY?

[ ] No [X] Yes; *please specify therapy and reaction:*

Itching and hives with fresh frozen plasma and cryoprecipitate

PHYSICAL EXAMINATION

Number and list each positive finding in pre-treatment examination. On post-treatment examination, use same number and indicate any changes in each finding. Use additional numbers to list any new positive findings.

Pre-Treatment	Post-Treatment

DIAGNOSIS

[X] Prophylaxis [ ] Joint Hemorrhage [ ] Muscle Hemorrhage

[ ] Overt Bleeding [ ] Massive Wound [ ] Surgery

CONCOMITANT MEDICATIONS
(In use at enrollment or begun during study)

DRUG	SINGLE DOSE	ROUTE (IM, IV, PO)	SCHEDULE	DATE STARTED	DATE STOPPED
None					

AP000742

ARMOUR000950

ARMO0000092_0164

Date	Hemoglobin	Hematocrit	HAA	Bilirubin	S.G.O.T.	
8/3/77	15.1	46.0	Neg.	.5	49	

AL-1259 ADMINISTRATION

Lot No. K852031 Dosage 3957 AHF units in 105 ml

AHF units/vial 1164 Solution Time 90 *(minutes)* Infusion Time 15 *(minutes)*

Calculated AHF Rise 107 % Actual AHF Rise 121 %

	Date	Time	B.P.	Pulse	Temp.	PTT	AHF%
Preinfusion	8/3		118/76	76			.01
15 minutes Immediate Post Infusion							1.21
1 hr " "							.99
3 hr " "							.86
6 hr " "							.81
9 hr " "							.65
12 hr " "							.35

ADVERSE EFFECT

[X] No adverse effects [ ] Adverse effects as in table:

SYMPTOMS OF SIGNS	DATE AND TIME OF ONSET	DURATION	SEVERITY*

*Classify reaction as 1 = mild; 2 = moderate; 3 = severe

Clinical Response The patient was not bleeding prior to infusion, therefore, clinical response could not be evaluated.

AP000743

_____, M.D. Date 9/13/77

Signature of Investigator

ANTIHEMOPHILIC FACTOR (HUMAN)
Protocol No. 101

162

Patient Identification [redacted] Study No. 1

Age 21 (yrs) Weight ____ (kg) or 180 (lbs) Height 72 (in) Case No. 10

MEDICAL HISTORY

Kidney Disease	[X] No	[ ] Yes	Hepatitis	[X] No	[ ] Yes	Date 8/3/77
Hypertension	[X] No	[ ] Yes	Jaundice	[X] No	[ ] Yes	Date 8/3/77
Uremia	[X] No	[ ] Yes	HAA Test	[X] Negative	[ ] Positive	Date 8/3/77

REACTIONS FROM *PREVIOUS* THERAPY?

[ ] No [X] Yes; *please specify therapy and reaction:*

Itching and hives with fresh frozen plasma and cryoprecipitate

PHYSICAL EXAMINATION

Number and list each positive finding in pre-treatment examination. On post-treatment examination, use same number and indicate any changes in each finding. Use additional numbers to list any new positive findings.

Pre-Treatment	Post-Treatment

DIAGNOSIS

[X] Prophylaxis [ ] Joint Hemorrhage [ ] Muscle Hemorrhage

[ ] Overt Bleeding [ ] Massive Wound [ ] Surgery

CONCOMITANT MEDICATIONS
(In use at enrollment or begun during study)

DRUG	SINGLE DOSE	ROUTE (IM, IV, PO)	SCHEDULE	DATE STARTED	DATE STOPPED
None					
			AP000744		

ARMOUR000952

Date	Hemoglobin	Hematocrit	HAA	Bilirubin	S.G.O.T.	
8/2/77	16.7	48.0	Neg.		47.0	

AL-1259 ADMINISTRATION

Lot No. K852031 Dosage 4375 AHF units in 90 ml

AHF units/vial 1164 Solution Time 60 *(minutes)* Infusion Time 15 *(minutes)*

Calculated AHF Rise 105 % Actual AHF Rise 117 %

	Date	Time	B.P.	Pulse	Temp.	PTT	AHF%
Preinfusion	8/2		125/84	70			<0.1 u/ml
15 minutes Immediate Post Infusion	8/2						1.17 u/ml
1 hr " "	8/2						1.00 u/ml
3 hr " "	8/2						.88 u/ml
6 hr " "	8/2						.79
9 hr " "	8/2						.68
12 hr " "	8/2						.39

ADVERSE EFFECT

[X] No adverse effects [ ] Adverse effects as in table:

SYMPTOMS OF SIGNS	DATE AND TIME OF ONSET	DURATION	SEVERITY*

*Classify reaction as 1 = mild; 2 = moderate; 3 = severe

Clinical Response The patient was not bleeding prior to infusion therefore, clinical response could not be evaluated.

AP000745

_____, M.D. Date 9/13/77

Signature of Investigator

1.4. STUDY 2 - Evaluation of AL-1259 by Dr. L. M.Aledort

Dr. Aledort studied eight patients with the established diagnosis of Haemophilia A. Four patients were treated for joint haemorrhages and four were prepared for various surgical procedures. In this study recovery of AHF activity in-vivo was poorer than in the other three studies, averaging 51% of the calculated values. Assay methods for AHF activity are known to vary considerably in different institutions. The investigator, who has a wide experience with AHF products, made no comment that recovery was unsatisfactory; hence it would appear that such recoveries are not unusual in his institution. In study 3, using the same lot of AL-1259 as study 2, much better recovery was observed. No adverse effects were observed in any of the eight patients in study 2. The clinical response was satisfactory in each case. Results are summarized overleaf.

STUDY 2 - LOUIS ALEDORT, M.D.

TABULATED RESULTS

Patient	Age	Wt. Kg.	Treatment	Dose* Units	Calculated Rise % AHF	Observed Rise % AHF	Adverse Effects
[redacted]	29	98	Surgery Preparation	3654	93	42	None
[redacted]	27	70	Surgery Preparation	2436	88	55	None
[redacted]	30	97	Surgery Preparation	4872	100	74	None
[redacted]	49	73	Joint Haemorrhage	2436	85	34.7	None
[redacted]	32	52	Joint Haemorrhage	1218	58	26	None
[redacted]	24	52	Joint Haemorrhage	1218	58	33.7	None
[redacted]	30	61	Surgery Preparation	2436	100	44.7	None
[redacted]	8	34	Joint Haemorrhage	1218	88	31.8	None

*AL-1259 Lot K852032, 1218 AHF units per vial

AP000747

165

ARMOUR000955

ARMO0000092_0169

AL-1259

ANTIHEMOPHILIC FACTOR (HUMAN)
Protocol No. 101

166

Patient Identification [redacted] Study No. 2

Age 29 *(yrs)* Weight 98 *(kg)* or ____ *(lbs)* Height 5'9" *(in)* Case No. 1

MEDICAL HISTORY

Kidney Disease	[X] No	[ ] Yes	Hepatitis	[X] No	[ ] Yes	Date ____
Hypertension	[X] No	[ ] Yes	Jaundice	[X] No	[ ] Yes	Date ____
Uremia	[X] No	[ ] Yes	HAA Test	[X] Negative	[ ] Positive	Date ____

REACTIONS FROM *PREVIOUS* THERAPY?

[ ] No [X] Yes; *please specify therapy and reaction:*

Urticaria p Cryoprecipitate and F.F.P. five years ago developed palpitations and tightness in chest to Factor VIII Concentrate

PHYSICAL EXAMINATION

Number and list each positive finding in pre-treatment examination. On post-treatment examination, use same number and indicate any changes in each finding. Use additional numbers to list any new positive findings.

Pre-Treatment	Post-Treatment
P.E. - WNL Exc. for flexion contracture	P.E. - unchanged
right knee	

DIAGNOSIS

[ ] Prophylaxis [ ] Joint Hemorrhage [ ] Muscle Hemorrhage

[ ] Overt Bleeding [ ] Massive Wound [X] Surgery

AP000748

CONCOMITANT MEDICATIONS
(In use at enrollment or begun during study)

DRUG	SINGLE DOSE	ROUTE *(IM, IV, PO)*	SCHEDULE	DATE STARTED	DATE STOPPED
BENADRYL	25 mg	I.V.	Prior to factor transfusion	10/19	10/19
DALMANE	30 mg	P.O.	QHS PRN	10/11	
CODEINE	30 mg	P.O.	Q3H PRN	10/11	
TYLENOL	5 gr (2)	P.O.	Q4H PRN	10/11	

ARMOUR000956

ARMO0000092_0170

Date	Hemoglobin	Hematocrit	HAA	Bilirubin	S.G.O.T.	167
10/11/77	14.0	43.3	NEG.	.5	21	

AL-1259 ADMINISTRATION

Lot No. K852032 Dosage 3654 AHF units in 90 ml

AHF units/vial 1218 Solution Time 50 *(minutes)* Infusion Time 40 *(minutes)*

Calculated AHF Rise 93 % Actual AHF Rise 42 %

	Date	Time	B.P.	Pulse	Temp:	PTT	AHF%
Preinfusion							<1%
Immediate Post Infusion							41.8%
1 hr " "							38.4%
4 hr " "							38.4%
12 hr " "							31.4%
24 hr " "							11.9%
__ hr " "							

ADVERSE EFFECT

[X] No adverse effects [ ] Adverse effects as in table:

SYMPTOMS OF SIGNS	DATE AND TIME OF ONSET	DURATION	SEVERITY*

*Classify reaction as 1 = mild; 2 = moderate; 3 = severe

Clinical Response Satisfactory

AP000749

_____, M.D. Date 10/19/77

Signature of Investigator

ARMOUR000957

ANTIHEMOPHILIC FACTOR (HUMAN)
Protocol No. 101

168

Patient Identification [redacted] Study No. 2

Age 27 (yrs) Weight 70 (kg) or ____ (lbs) Height 5'9" (in) Case No. 2

MEDICAL HISTORY

Kidney Disease	[XX] No	[ ] Yes	Hepatitis	[X] No	[ ] Yes	Date	
Hypertension	[X] No	[ ] Yes	Jaundice	[X] No	[ ] Yes	Date	
Uremia	[X] No	[ ] Yes	HAA Test	[X] Negative	[ ] Positive	Date	10/5/77

REACTIONS FROM *PREVIOUS* THERAPY?

[X] No [ ] Yes; *please specify therapy and reaction:*

PHYSICAL EXAMINATION

Number and list each positive finding in pre-treatment examination. On post-treatment examination, use same number and indicate any changes in each finding. Use additional numbers to list any new positive findings.

Pre-Treatment	Post-Treatment
P.E. WNL Exc. for S_4 gallop and right	P.E. unchanged
knee deformity and contracture	

DIAGNOSIS

[ ] Prophylaxis [ ] Joint Hemorrhage [ ] Muscle Hemorrhage

[ ] Overt Bleeding [ ] Massive Wound [X] Surgery

CONCOMITANT MEDICATIONS
(In use at enrollment or begun during study)

AP000750

DRUG	SINGLE DOSE	ROUTE (IM, IV, PO)	SCHEDULE	DATE STARTED	DATE STOPPED
DILANTIN	100 mg	P.O.	T.I.D.	10/1	
ELAVIL	150 mg	P.O.	G.H.S.	10/14	
DEMEROL	50 mg	P.O.	Q6H PRN pain	10/16	

ARMOUR000958

ARMO0000092_0172

Date	Hemoglobin	Hematocrit	HAA	Bilirubin	S.G.O.T.
10/18/77	15.3	43.8	NEG.	.4	39

AL-1259 ADMINISTRATION

Lot No. K852032 Dosage 2436 AHF units in 60 ml

AHF units/vial 1218 Solution Time 50 *(minutes)* Infusion Time 30 *(minutes)*

Calculated AHF Rise 88 % Actual AHF Rise 55 %

	Date	Time	B.P.	Pulse	Temp.	PTT	AHF%
Preinfusion							<1%
Immediate Post Infusion							55%
1 hr " "							46.1%
4 hr " "							37.1%
12 hr " "							28%
24 hr " "							9.2%
__ hr " "							

ADVERSE EFFECT

☒ No adverse effects ☐ Adverse effects as in table:

SYMPTOMS OF SIGNS	DATE AND TIME OF ONSET	DURATION	SEVERITY*

*Classify reaction as 1 = mild; 2 = moderate; 3 = severe

Clinical Response Satisfactory

AP000751

_____, M.D. Date 10/19/77

Signature of Investigator

ANTIHEMOPHILIC FACTOR (HUMAN)
Protocol No. 101

Patient Identification [redacted] Study No. 2

Age 30 (yrs) Weight ____ (kg) or 211 (lbs) Height 5'8" (in) Case No. 3

MEDICAL HISTORY

Kidney Disease	[X] No	[ ] Yes	Hepatitis	[X] No	[ ] Yes	Date ____
Hypertension	[X] No	[ ] Yes	Jaundice	[X] No	[ ] Yes	Date ____
Uremia	[X] No	[ ] Yes	HAA Test	[X] Negative	[ ] Positive	Date 9/16/77

REACTIONS FROM *PREVIOUS* THERAPY?

[X] No [ ] Yes; *please specify therapy and reaction:*

PHYSICAL EXAMINATION

Number and list each positive finding in pre-treatment examination. On post-treatment examination, use same number and indicate any changes in each finding. Use additional numbers to list any new positive findings.

Pre-Treatment	Post-Treatment
1. Right femoral pseudo tumor	1. no change

DIAGNOSIS

[ ] Propnylaxis [ ] Joint Hemorrhage [ ] Muscle Hemorrhage

[ ] Overt Bleeding [ ] Massive Wound [X] Surgery

CONCOMITANT MEDICATIONS
(In use at enrollment or begun during study)

AP000752

DRUG	SINGLE DOSE	ROUTE (IM, IV, PO)	SCHEDULE	DATE STARTED	DATE STOPPED
Colace	300 mg	PO	QID	9/14/77	12/9/77
Valium	5 mg	PO	Q6H	9/12/77	12/9/77
Tylenol	650 mg	PO	Q4H	9/12/77	12/9/77

Date	Hemoglobin	Hematocrit	HAA	Bilirubin	S.G.O.T.	171
9/12/77	14.5	43.9	(--)	.9	17	
11/12/77	13.1	38.3	(--)	1.0	17	

AL-1259 ADMINISTRATION

Lot No. K852032 Dosage 4872 AHF units in 200 ml

AHF units/vial 1218 Solution Time 40 *(minutes)* Infusion Time 30 *(minutes)*

Calculated AHF Rise 100 % Actual AHF Rise 74 %

11/16/77	Date	Time	B.P.	Pulse	Temp.	PTT	AHF%
Preinfusion							<1%
Immediate Post Infusion							74%
1 hr " "							Clotted
4 hr " "							57.1%
12 hr " "							34.7%
24 hr " "							12.8%
__ hr " "							

ADVERSE EFFECT

[X] No adverse effects [ ] Adverse effects as in table:

SYMPTOMS OF SIGNS	DATE AND TIME OF ONSET	DURATION	SEVERITY*

*Classify reaction as 1 = mild; 2 = moderate; 3 = severe

Clinical Response Satisfactory

AP000753

_____, M.D. Date 2/20/78

Signature of Investigator

ARMOUR000961

AL-1259
ANTIHEMOPHILIC FACTOR (HUMAN)
Protocol No. 101

172

Patient Identification [redacted] Study No. 2

Age 49 *(yrs)* Weight ____ *(kg)* or 160 *(lbs)* Height 5'11" *(in)* Case No. 4

MEDICAL HISTORY

Kidney Disease	[X] No	[ ] Yes	Hepatitis	[ ] No	[X] Yes	Date 1972
Hypertension	[ ] No	[X] Yes	Jaundice	[X] No	[ ] Yes	Date ____
Uremia	[X] No	[ ] Yes	HAA Test	[X] Negative	[ ] Positive	Date 1/21/77

REACTIONS FROM *PREVIOUS* THERAPY?

[X] No [ ] Yes; *please specify therapy and reaction:*

PHYSICAL EXAMINATION

Number and list each positive finding in pre-treatment examination. On post-treatment examination, use same number and indicate any changes in each finding. Use additional numbers to list any new positive findings.

Pre-Treatment	Post-Treatment
1. Deformity and Swelling R. Knee and L. Knee	1. Decreased swelling of R. Knee

DIAGNOSIS

[ ] Prophylaxis [X] Joint Hemorrhage [ ] Muscle Hemorrhage
[ ] Overt Bleeding [ ] Massive Wound [ ] Surgery

CONCOMITANT MEDICATIONS
(In use at enrollment or begun during study)

DRUG	SINGLE DOSE	ROUTE *(IM, IV, PO)*	SCHEDULE	DATE STARTED	DATE STOPPED
Hydrodiurel	50 mg	PO	BID		
Librium	25 mg	PO	QHS		

AP000754

Date	Hemoglobin	Hematocrit	HAA	Bilirubin	S.G.O.T.	173
11/14/77	15.6	45.0	Neg.	.3	32	

AL-1259 ADMINISTRATION

Lot No. K852032 Dosage 2436 AHF units in 60 ml

AHF units/vial 1218 Solution Time ____ *(minutes)* Infusion Time ____ *(minutes)*

Calculated AHF Rise 85 % Actual AHF Rise 34.7 %

11/4/77 Date	Time	B.P.	Pulse	Temp.	PTT	AHF%
Preinfusion						1.6%
Immediate Post Infusion						36.3%
1 hr " "						
4 hr " "						
12 hr " "						
24 hr " "						
__ hr " "						

ADVERSE EFFECT

☒ No adverse effects ☐ Adverse effects as in table:

SYMPTOMS OF SIGNS	DATE AND TIME OF ONSET	DURATION	SEVERITY*

*Classify reaction as 1 = mild; 2 = moderate; 3 = severe

Clinical Response Satisfactory

AP000755

_____, M.D. Date 2/25/78

Signature of Investigator

ARMOUR000963

AL-1254

ANTIHEMOPHILIC FACTOR (HUMAN)
Protocol No. 101

174

Patient Identification [redacted] Study No. 2

Age 32 *(yrs)* Weight ____ *(kg)* or 115 *(lbs)* Height 5'5" *(in)* Case No. 5

MEDICAL HISTORY

Kidney Disease	[X] No	[ ] Yes	Hepatitis	[X] No	[ ] Yes	Date	
Hypertension	[X] No	[ ] Yes	Jaundice	[X] No	[ ] Yes	Date	
Uremia	[X] No	[ ] Yes	HAA Test	[X] Negative	[ ] Positive	Date	11/4/77

REACTIONS FROM *PREVIOUS* THERAPY?

[X] No [ ] Yes; *please specify therapy and reaction:*

PHYSICAL EXAMINATION

Number and list each positive finding in pre-treatment examination. On post-treatment examination, use same number and indicate any changes in each finding. Use additional numbers to list any new positive findings.

Pre-Treatment	Post-Treatment
1. Chronic deformity and limited R.O.M. of	1. No change
L. Elbow, R. Knee and R. Shoulder	

DIAGNOSIS

[X] Prophylaxis [ ] Joint Hemorrhage [ ] Muscle Hemorrhage

[ ] Overt Bleeding [ ] Massive Wound [ ] Surgery

CONCOMITANT MEDICATIONS
(In use at enrollment or begun during study)

DRUG	SINGLE DOSE	ROUTE *(IM, IV, PO)*	SCHEDULE	DATE STARTED	DATE STOPPED
None					

AP000756

ARMOUR000964

Date	Hemoglobin	Hematocrit	HAA	Bilirubin	S.G.O.T.	175
11/ 77			(--)			

AL-1259 ADMINISTRATION

Lot No. K852032 Dosage 1218 AHF units in 30 ml

AHF units/vial 1218 Solution Time 30 *(minutes)* Infusion Time 17 *(minutes)*

Calculated AHF Rise 58 % Actual AHF Rise 26 %

	11/4/77 Date	Time	B.P.	Pulse	Temp.	PTT	AHF%
Preinfusion							<1%
Immediate Post Infusion							26%
1 hr " "							
4 hr " "							
12 hr " "							
24 hr " "							
__ hr " "							

ADVERSE EFFECT

[X] No adverse effects [ ] Adverse effects as in table:

SYMPTOMS OF SIGNS	DATE AND TIME OF ONSET	DURATION	SEVERITY*

*Classify reaction as 1 = mild; 2 = moderate; 3 = severe

Clinical Response Satisfactory

AP000757

_____, M.D. Date 2/20/78

Signature of Investigator

ARMOUR000965

AL-1259
ANTIHEMOPHILIC FACTOR (HUMAN)
Protocol No. 101

176

Patient Identification [redacted] Study No. 2

Age 24 (yrs) Weight ____ (kg) or 115 (lbs) Height ____ (in) Case No. 6

MEDICAL HISTORY

Kidney Disease	[X] No	[ ] Yes	Hepatitis	[X] No	[ ] Yes	Date ____
Hypertension	[X] No	[ ] Yes	Jaundice	[X] No	[ ] Yes	Date ____
Uremia	[X] No	[ ] Yes	HAA Test	[X] Negative	[ ] Positive	Date 8/12/77

REACTIONS FROM *PREVIOUS* THERAPY?

[X] No [ ] Yes; *please specify therapy and reaction:*

PHYSICAL EXAMINATION

Number and list each positive finding in pre-treatment examination. On post-treatment examination, use same number and indicate any changes in each finding. Use additional numbers to list any new positive findings.

Pre-Treatment	Post-Treatment
1. Mild Bi-laterial Knee Deformity	1. No change

DIAGNOSIS

[X] Prophylaxis [ ] Joint Hemorrhage [ ] Muscle Hemorrhage

[ ] Overt Bleeding [ ] Massive Wound [ ] Surgery

CONCOMITANT MEDICATIONS
(In use at enrollment or begun during study)

DRUG	SINGLE DOSE	ROUTE (IM, IV, PO)	SCHEDULE	DATE STARTED	DATE STOPPED
None					
			AP000758		

Date	Hemoglobin	Hematocrit	HAA	Bilirubin	S.G.O.T.	177
8/ /77	15.5	45.1	Neg.	.7	27	

AL-1259 ADMINISTRATION

Lot No. K852032 Dosage 1218 AHF units in 30 ml

AHF units/vial 1218 Solution Time 32' *(minutes)* Infusion Time 20 *(minutes)*

Calculated AHF Rise 58 % Actual AHF Rise 33.7 %

	11/11/77 Date	Time	B.P.	Pulse	Temp.	PTT	AHF%
Preinfusion	11/11						1.7%
Immediate Post Infusion							35.4%
1 hr " "							
4 hr " "							
12 hr " "							
24 hr " "							
__ hr " "							

ADVERSE EFFECT

[X] No adverse effects [ ] Adverse effects as in table:

SYMPTOMS OF SIGNS	DATE AND TIME OF ONSET	DURATION	SEVERITY*

*Classify reaction as 1 = mild; 2 = moderate; 3 = severe

Clinical Response Satisfactory

AP000759

_____, M.D. Date 2/20/78

Signature of Investigator

ANTIHEMOPHILIC FACTOR (HUMAN)
Protocol No. 101

Patient Identification [redacted] Study No. 2

Age 30 (yrs) Weight ____ (kg) or 135 (lbs) Height 5'8" (in) Case No. 7

MEDICAL HISTORY

Kidney Disease	[X] No	[ ] Yes	Hepatitis	[ ] No	[ ] Yes	Date ____
Hypertension	[X] No	[ ] Yes	Jaundice	[ ] No	[ ] Yes	Date ____
Uremia	[X] No	[ ] Yes	HAA Test	[X] Negative	[ ] Positive	Date 9/23/77

REACTIONS FROM *PREVIOUS* THERAPY?

[ ] No [X] Yes; *please specify therapy and reaction:*

Chills and mild Urticaria

PHYSICAL EXAMINATION

Number and list each positive finding in pre-treatment examination. On post-treatment examination, use same number and indicate any changes in each finding. Use additional numbers to list any new positive findings.

Pre-Treatment	Post-Treatment
1. Knee Deformity, R. more than L.	1. No change
2. Spleen tip palpable	

DIAGNOSIS

[ ] Prophylaxis [ ] Joint Hemorrhage [ ] Muscle Hemorrhage

[ ] Overt Bleeding [ ] Massive Wound [X] Surgery

CONCOMITANT MEDICATIONS
(In use at enrollment or begun during study)

DRUG	SINGLE DOSE	ROUTE (IM, IV, PO)	SCHEDULE	DATE STARTED	DATE STOPPED
Benadryl	25 mg	IV	Stat	11/21/77	11/21/77
			AP000760		

Date	Hemoglobin	Hematocrit	HAA	Bilirubin	S.G.O.T.	
4/11/77	15.7	49.1	(--)	1.3	33	

AL-1259 ADMINISTRATION

Lot No. K852032 Dosage 2436 AHF units in 60 ml

AHF units/vial 1218 Solution Time _____ *(minutes)* Infusion Time _____ *(minutes)*

Calculated AHF Rise 100 % Actual AHF Rise 44.7 %

11/21/77	Date	Time	B.P.	Pulse	Temp.	PTT	AHF%
Preinfusion							7.6%
Immediate Post Infusion							52.3%
1 hr " "							
4 hr " "							
12 hr " "							
24 hr " "							
__ hr " "							

ADVERSE EFFECT

[X] No adverse effects [ ] Adverse effects as in table:

SYMPTOMS OF SIGNS	DATE AND TIME OF ONSET	DURATION	SEVERITY*

*Classify reaction as1 = mild; 2 = moderate; 3 = severe

Clinical Response Satisfactory

AP000761

_____, M.D. Date 2/20/78

Signature of Investigator

ARMOUR000969

AL-1259
ANTIHEMOPHILIC FACTOR (HUMAN)
Protocol No. 101

180

Patient Identification [redacted] Study No. 2

Age 8 (yrs) Weight ____ (kg) or 75 (lbs) Height 4'3" (in) Case No. 8

MEDICAL HISTORY

Kidney Disease	[X] No	[ ] Yes	Hepatitis	[X] No	[ ] Yes	Date ____
Hypertension	[X] No	[ ] Yes	Jaundice	[X] No	[ ] Yes	Date ____
Uremia	[X] No	[ ] Yes	HAA Test	[X] Negative	[ ] Positive	Date ____

REACTIONS FROM *PREVIOUS* THERAPY?

[ ] No [X] Yes; *please specify therapy and reaction:*

Reaction to Factor VIII Concentrate four years ago

PHYSICAL EXAMINATION

Number and list each positive finding in pre-treatment examination. On post-treatment examination, use same number and indicate any changes in each finding. Use additional numbers to list any new positive findings.

Pre-Treatment	Post-Treatment
1. Swollen R. Ankle	1. Decrease in Warmth and Swelling of Ankle

DIAGNOSIS

[ ] Prophylaxis [X] Joint Hemorrhage [ ] Muscle Hemorrhage

[ ] Overt Bleeding [ ] Massive Wound [ ] Surgery

CONCOMITANT MEDICATIONS
(In use at enrollment or begun during study)

DRUG	SINGLE DOSE	ROUTE (IM, IV, PO)	SCHEDULE	DATE STARTED	DATE STOPPED
None					

AP000762

ARMOUR000970

ARMO0000092_0184

1.5. STUDY 3 - Evaluation of AL-1259 by Dr. M. W. Hilgartner

Dr. Hilgartner studied 3 paediatric and 2 adult patients with the established diagnosis of Haemophilia A. One paediatric patient was treated on two occasions of joint haemorrhage, one paediatric patient on one occasion of joint haemorrhage, and the third paediatric patient received prophylactic treatment. One adult patient was prepared for surgery and the other received prophylactic treatment. On the first treatment of patient MC, the in-vivo recovery of AHF activity was poor. It is possible that an undetected low titre inhibitor of AHF may have been present; this has been reported in as many as 20% of haemophilic patients. Leaving this one treatment out of consideration, recovery of AHF in-vivo in the five patients averaged 83% of the expected values. No adverse effects were observed. The results of this study are tabulated overleaf.

Date	Hemoglobin	Hematocrit	HAA	Bilirubin	S.G.O.T.	181
/ /77			Neg.	1.4	43	

AL-1259 ADMINISTRATION

Lot No. K852032 Dosage 1218 AHF units in 30 ml

AHF units/vial 1218 Solution Time _____ *(minutes)* Infusion Time _____ *(minutes)*

Calculated AHF Rise 88 % Actual AHF Rise 31.8 %

	11/23/77 Date	Time	B.P.	Pulse	Temp.	PTT	AHF%
Preinfusion	11/23						4.8%
Immediate Post Infusion							36.6%
1 hr " "							
4 hr " "							
12 hr " "							
24 hr " "							
__ hr " "							

ADVERSE EFFECT

☒ No adverse effects ☐ Adverse effects as in table:

SYMPTOMS OF SIGNS	DATE AND TIME OF ONSET	DURATION	SEVERITY*

*Classify reaction as 1 = mild; 2 = moderate; 3 = severe

Clinical Response Satisfactory

AP000763

_____, M.D. Date 2/20/78

Signature of Investigator

ARMOUR000972

STUDY 3 - MARGARET W. HILGARTNER, M.D.

TABULATED RESULTS

Patient	Age	Wt. Kg.	Treatment	Dose* Units	Calculated Rise % AHF	Observed Rise % AHF	Adverse Effects
[redacted]	27	90.5	Surgery Preparation	2436	54	44	None
[redacted]	15	46.4	Joint Haemorrhage	2436	105	88	None
[redacted]	25	85.5	Prophylactic	2436	57	31	None
[redacted]	7	24.5	Joint Haemorrhage 11-4 Joint Haemorrhage 12-15	2436 1218	191** 96	94** 98	None None
[redacted]	17	70.5	Prophylactic	1218	35	33	None

*AL-1259 Lot K852032, 1218 AHF units per vial
**Value not included in average. See explanation, report of evaluation, Study 3

AP000765

183

ARMOUR000973

ARMO0000092_0187

AL-1259

ANTIHEMOPHILIC FACTOR (HUMAN)
Protocol No. 101

184

Patient Identification [redacted] Study No. 3

Age 27 (yrs) Weight 90.5 (kg) or ____ (lbs) Height 70½ (in) Case No. 1

MEDICAL HISTORY

Kidney Disease	☐ No	☒ Yes	Hepatitis	☒ No	☐ Yes	Date ____
Hypertension	☒ No	☐ Yes	Jaundice	☒ No	☐ Yes	Date ____
Uremia	☒ No	☐ Yes	HB Ag HAA Test	☒ Negative	☐ HB AG Pos Positive	Date ____

REACTIONS FROM *PREVIOUS* THERAPY?

☐ No ☒ Yes; *please specify therapy and reaction:*

FFP - fever

PHYSICAL EXAMINATION

Number and list each positive finding in pre-treatment examination. On post-treatment examination, use same number and indicate any changes in each finding. Use additional numbers to list any new positive findings.

Pre-Treatment	Post-Treatment
Pre op study	

DIAGNOSIS

☐ Prophylaxis ☐ Joint Hemorrhage ☐ Muscle Hemorrhage

☐ Overt Bleeding ☐ Massive Wound ☒ Surgery

CONCOMITANT MEDICATIONS
(In use at enrollment or begun during study)

DRUG	SINGLE DOSE	ROUTE (IM, IV, PO)	SCHEDULE	DATE STARTED	DATE STOPPED
~~0~~					
			AP000766		

ARMOUR000974

ARMO0000092_0188

Date	Hemoglobin	Hematocrit	HAA	Bilirubin	S.G.O.T.	185
10/. /77	16.1	45.2	Neg.	1.0	50	

AL-1259 ADMINISTRATION

Lot No. 1259 Dosage 2436 AHF units in 60 ml

AHF units/vial 1218 Solution Time _____ *(minutes)* Infusion Time _____ *(minutes)*

Calculated AHF Rise 53.8 % Actual AHF Rise 43.6 %

	Date	Time	B.P.	Pulse	Temp.	PTT	AHF%
Preinfusion	10/21/77					64.9	less than 1%
(½ hour) Immediate Post Infusion	10/21/77						43.7
1 hr " "	10/21/77						38.8
3 hr 4 hr " "	10/21/77						21.6
6 hr 12 hr " "	10/21/77						19.7
24 hr " "	10/22/77						6.7
__ hr " "							

ADVERSE EFFECT

[XX] No adverse effects [ ] Adverse effects as in table:

SYMPTOMS OF SIGNS	DATE AND TIME OF ONSET	DURATION	SEVERITY*

*Classify reaction as 1 = mild; 2 = moderate; 3 = severe

Clinical Response _____

AP000767

_____, M.D. Date 2/9/78

Signature of Investigator

ARMOUR000975

ARMO0000092_0189

ANTIHEMOPHILIC FACTOR (HUMAN)
Protocol No. 101

186

Patient Identification [redacted] Study No. 3

Age 15 (yrs) Weight 46.36 (kg) or ____ (lbs) Height 66 (in) Case No. 2

MEDICAL HISTORY

Kidney Disease	[X] No	[ ] Yes	Hepatitis	[X] No	[ ] Yes	Date
Hypertension	[X] No	[ ] Yes	Jaundice	[X] No	[ ] Yes	Date
Uremia	[X] No	[ ] Yes	HAA Test	[ ] Negative	[X] Positive (HB AB)	Date

REACTIONS FROM *PREVIOUS* THERAPY?

[ ] No [X] Yes; *please specify therapy and reaction:*

FFP Hd B_s

PHYSICAL EXAMINATION

Number and list each positive finding in pre-treatment examination. On post-treatment examination, use same number and indicate any changes in each finding. Use additional numbers to list any new positive findings.

Pre-Treatment	Post-Treatment
Rt Knee swollen, warm, tender	swollen, warm, less tender
Flexion contracture	same

DIAGNOSIS

[ ] Prophylaxis [X] Joint Hemorrhage [ ] Muscle Hemorrhage

[ ] Overt Bleeding [ ] Massive Wound [ ] Surgery

CONCOMITANT MEDICATIONS
(In use at enrollment or begun during study)

DRUG	SINGLE DOSE	ROUTE (IM, IV, PO)	SCHEDULE	DATE STARTED	DATE STOPPED
0					
			AP000768		

ARMOUR000976

ARMO0000092_0190

Date	Hemoglobin	Hematocrit	HAA	Bilirubin	S.G.O.T.	T8?
/ /77	14.3	45.3	Neg.	0.6	90	

AL-1259 ADMINISTRATION

Lot No. 1259 Dosage 2436 AHF units in 60 ml

AHF units/vial 1218 Solution Time _____ *(minutes)* Infusion Time _____ *(minutes)*

Calculated AHF Rise 105.2 % Actual AHF Rise 87.9 %

	Date	Time	B.P.	Pulse	Temp.	PTT	AHF%
Preinfusion	10/25/77					71.4	3.4
Immediate Post Infusion							
½ hr ~~xxx~~ " "	10/25/77						91.3
4 hr " "							
12 hr " "							
24 hr " "							
__ hr " "							

ADVERSE EFFECT

[X] No adverse effects [ ] Adverse effects as in table:

SYMPTOMS OF SIGNS	DATE AND TIME OF ONSET	DURATION	SEVERITY*

*Classify reaction as 1 = mild; 2 = moderate; 3 = severe

Clinical Response _____

AP000769

_____, M.D. Date 2/9/78

Signature of Investigator

ANTIHEMOPHILIC FACTOR (HUMAN)
Protocol No. 101

Patient Identification [redacted] Study No. 3

Age 25 (yrs) Weight 85.45 (kg) or ____ (lbs) Height 73 (in) Case No. 3

MEDICAL HISTORY

Kidney Disease	[X] No	[ ] Yes	Hepatitis	[X] No	[ ] Yes	Date
Hypertension	[X] No	[ ] Yes	Jaundice	[X] No	[ ] Yes	Date
Uremia	[X] No	[ ] Yes	HAA Test	[X] Negative	[ ] Positive	Date

REACTIONS FROM *PREVIOUS* THERAPY?

[ ] No [X] Yes, *please specify therapy and reaction:*

FFP cryo, hives, fever

PHYSICAL EXAMINATION

Number and list each positive finding in pre-treatment examination. On post-treatment examination, use same number and indicate any changes in each finding. Use additional numbers to list any new positive findings.

Pre-Treatment	Post-Treatment

DIAGNOSIS

[X] Prophylaxis [ ] Joint Hemorrhage [ ] Muscle Hemorrhage
[ ] Overt Bleeding [ ] Massive Wound [ ] Surgery

CONCOMITANT MEDICATIONS
(In use at enrollment or begun during study)

DRUG	SINGLE DOSE	ROUTE (IM, IV, PO)	SCHEDULE	DATE STARTED	DATE STOPPED
0					

AP000770

ARMOUR000978

ARMO0000092_0192

Date	Hemoglobin	Hematocrit	HAA	Bilirubin	S.G.O.T.	189
/ /77	16.2	47.5	Neg.	0.5	39	

AL-1259 ADMINISTRATION

Lot No. 1259 Dosage 2436 AHF units in 60 ml

AHF units/vial 1218 Solution Time _____ *(minutes)* Infusion Time _____ *(minutes)*

Calculated AHF Rise 57 % Actual AHF Rise 30.6 %

	Date	Time	B.P.	Pulse	Temp.	PTT	AHF%
Preinfusion	11/4/77					74.5	Less than 1%
Immediate Post Infusion							
½ hr " "	11/4/77						30.6
4 hr " "							
12 hr " "							
24 hr " "							
__ hr " "							

ADVERSE EFFECT

[X] No adverse effects [ ] Adverse effects as in table:

SYMPTOMS OF SIGNS	DATE AND TIME OF ONSET	DURATION	SEVERITY*

*Classify reaction as 1 = mild; 2 = moderate; 3 = severe

Clinical Response _____

AP000771

_____, M.D. Date 2/9/78

Signature of Investigator

ARMOUR000979

Protocol No. 101

190

Patient Identification [redacted] Study No. 3

Age 7 (yrs) Weight 25.45 (kg) or ____ (lbs) Height 50 (in) Case No. 4

MEDICAL HISTORY

Kidney Disease	[X] No	[ ] Yes	Hepatitis	[X] No	[ ] Yes	Date ____
Hypertension	[X] No	[ ] Yes	Jaundice	[X] No	[ ] Yes	Date ____
Uremia	[X] No	[ ] Yes	HAA Test	[X] Negative	[ ] Positive	Date ____

REACTIONS FROM *PREVIOUS* THERAPY?

[X] No [ ] Yes; *please specify therapy and reaction:*

PHYSICAL EXAMINATION

Number and list each positive finding in pre-treatment examination. On post-treatment examination, use same number and indicate any changes in each finding. Use additional numbers to list any new positive findings.

Pre-Treatment	Post-Treatment
Sinovitis L Knee →	
Swollen, tender ↓ ROM	

DIAGNOSIS

[ ] Prophylaxis [X] Joint Hemorrhage [ ] Muscle Hemorrhage

[ ] Overt Bleeding [ ] Massive Wound [ ] Surgery

CONCOMITANT MEDICATIONS
(In use at enrollment or begun during study)

DRUG	SINGLE DOSE	ROUTE (IM, IV, PO)	SCHEDULE	DATE STARTED	DATE STOPPED
0					
			AP000772		

ARMOUR000980

Date	Hemoglobin	Hematocrit	HAA	Bilirubin	S.G.O.T.	191
11/4/77	12.1	33.5	Neg.	0.4	180	

AL-1259 ADMINISTRATION

Lot No. 1259 Dosage 2436 AHF units in 60 ml

AHF units/vial 1218 Solution Time _____ *(minutes)* Infusion Time _____ *(minutes)*

Calculated AHF Rise 191 % Actual AHF Rise 94 %

	Date	Time	B.P.	Pulse	Temp.	PTT	AHF%
Preinfusion	11/4/77					80	Less than 1%
Immediate Post Infusion							
½ hr " "	11/4/77						94
4 hr " "							
12 hr " "							
24 hr " "							
__ hr " "							

ADVERSE EFFECT

[X] No adverse effects [ ] Adverse effects as in table:

SYMPTOMS OF SIGNS	DATE AND TIME OF ONSET	DURATION	SEVERITY*

*Classify reaction as 1 = mild; 2 = moderate; 3 = severe

Clinical Response _____

AP000773

_____, M.D. Date 2/3/78

Signature of Investigator

ARMOUR000981

Protocol No. 101

197

Patient Identification [redacted] Study No. 3

Age 7 (yrs) Weight 25.45(kg) or ____ (lbs) Height 50 (in) Case No. 5

MEDICAL HISTORY

Kidney Disease	[X] No	[ ] Yes	Hepatitis	[X] No	[ ] Yes	Date ____
Hypertension	[X] No	[ ] Yes	Jaundice	[X] No	[ ] Yes	Date ____
Uremia	[X] No	[ ] Yes	HAA Test	[X] Negative	[ ] Positive	Date ____

REACTIONS FROM *PREVIOUS* THERAPY?

[X] No [ ] Yes; *please specify therapy and reaction:*

PHYSICAL EXAMINATION

Number and list each positive finding in pre-treatment examination. On post-treatment examination, use same number and indicate any changes in each finding. Use additional numbers to list any new positive findings.

Pre-Treatment	Post-Treatment
Sinovitis L Knee →	→
swollen, tender ↓ ROM	

DIAGNOSIS

[ ] Prophylaxis [X] Joint Hemorrhage [ ] Muscle Hemorrhage

[ ] Overt Bleeding [ ] Massive Wound [ ] Surgery

CONCOMITANT MEDICATIONS
(In use at enrollment or begun during study)

DRUG	SINGLE DOSE	ROUTE (IM, IV, PO)	SCHEDULE	DATE STARTED	DATE STOPPED
0					
			AP000774		

Date	Hemoglobin	Hematocrit	HAA	Bilirubin	S.G.O.T.	
11/4/77	12.1	33.5	Neg.	0.4	180	193

AL-1259 ADMINISTRATION

Lot No. 1259 Dosage 1218 AHF units in 30 ml

AHF units/vial 1218 Solution Time _____ *(minutes)* Infusion Time _____ *(minutes)*

Calculated AHF Rise 95.7 % Actual AHF Rise 98 %

	Date	Time	B.P.	Pulse	Temp.	PTT	AHF%
Preinfusion	12/15/77						less than 1%
Immediate Post Infusion							
½ hr XXXX " "							98
4 hr " "							
12 hr " "							
24 hr " "							
__ hr " "							

ADVERSE EFFECT

[X] No adverse effects [ ] Adverse effects as in table:

SYMPTOMS OF SIGNS	DATE AND TIME OF ONSET	DURATION	SEVERITY*

*Classify reaction as 1 = mild; 2 = moderate; 3 = severe

Clinical Response _____

AP000775

_____, M.D. Date 2/9/78

Signature of Investigator

ARMOUR000983

AL-1259

ANTIHEMOPHILIC FACTOR (HUMAN)
Protocol No. 101

194

Patient Identification [redacted] Study No. 3

Age 17 *(yrs)* Weight 70.45 *(kg)* or ____ *(lbs)* Height 68 *(in)* Case No. 6

MEDICAL HISTORY

Kidney Disease	[X] No	[ ] Yes	Hepatitis	[X] No	[ ] Yes	Date ____
Hypertension	[X] No	[ ] Yes	Jaundice	[X] No	[ ] Yes	Date ____
Uremia	[X] No	[ ] Yes	HAA Test	[X] Negative	[ ] Positive	Date ____

REACTIONS FROM *PREVIOUS* THERAPY?

[ ] No [X] Yes; *please specify therapy and reaction:*

FFP - fever

PHYSICAL EXAMINATION

Number and list each positive finding in pre-treatment examination. On post-treatment examination, use same number and indicate any changes in each finding. Use additional numbers to list any new positive findings.

Pre-Treatment	Post-Treatment

DIAGNOSIS

[X] Prophylaxis [ ] Joint Hemorrhage [ ] Muscle Hemorrhage

[ ] Overt Bleeding [ ] Massive Wound [ ] Surgery

CONCOMITANT MEDICATIONS
(In use at enrollment or begun during study)

DRUG	SINGLE DOSE	ROUTE *(IM, IV, PO)*	SCHEDULE	DATE STARTED	DATE STOPPED

AP000776

ARMOUR000984

Date	Hemoglobin	Hematocrit	HAA	Bilirubin	S.G.O.T.	195
12/ /77	18.6	47.6	Neg.	0.9	30	

AL-1259 ADMINISTRATION

Lot No. 1259 Dosage 1218 AHF units in 30 ml

AHF units/vial 1218 Solution Time _____ *(minutes)* Infusion Time _____ *(minutes)*

Calculated AHF Rise 35 % Actual AHF Rise 33 %

	Date	Time	B.P.	Pulse	Temp.	PTT	AHF%
Preinfusion	12/19/77						less than 1%
Immediate Post Infusion							
½ hr " "	12/19/77						33
4 hr " "							
12 hr " "							
24 hr " "							
__ hr " "							

ADVERSE EFFECT

[x] No adverse effects [ ] Adverse effects as in table:

SYMPTOMS OF SIGNS	DATE AND TIME OF ONSET	DURATION	SEVERITY*

*Classify reaction as 1 = mild; 2 = moderate; 3 = severe

Clinical Response _____

AP000777

_____, M.D. Date 2/9/78

Signature of Investigator

1.6. STUDY 4 - Evaluation of AL-1259 by Dr. P. H. Levine

Dr. Levine studied one paediatric and four adult patients with established diagnosis of Haemophilia A. Two patients were treated for joint haemorrhage and one received prophylactic treatment. One patient was prepared for arthroscopy and one received pre and post-operative treatment in a total hip replacement. The clinical response in the latter case was deemed unusually good in that this major operation was uneventful and there was no post-operative bleeding. No adverse effects were observed in any of the five patients. Recovery of AHF activity in-vivo averaged 116% of the calculated value. The clinical response was satisfactory in each case. The results of this study are tabulated overleaf.

STUDY 4 - PETER H. LEVINE, M.D.

TABULATED RESULTS

Patient	Age	Wt. Kg.	Treatment	Dose* Units	Calculated Rise % AHF	Observed Rise % AHF	Adverse Effects
[redacted]	28	70	Surgery Preparation	3942	100	117	None
[redacted]	38	85	Surgery Preparation	2628	66	72	None
			Post Operative	1314	32	42	None
[redacted]	13	30	Joint Haemorrhage	1314	88	86	None
[redacted]	24	54	Joint Haemorrhage	1314	48	55	None
[redacted]	33	71	Prophylactic	1314	38	49	None

*AL-1259 Lot K852030, 1314 AHF units per vial

AP000779

197

ARMOUR000987

ARMO0000092_0201

ANTIHEMOPHILIC FACTOR (HUMAN)
Protocol No. 101

198

Patient Identification [redacted] Study No. 4

Age 28 (yrs) Weight 70 (kg) or ____ (lbs) Height 65 (in) Case No. 1

MEDICAL HISTORY

Kidney Disease	☒ No	☐ Yes	Hepatitis	☒ No	☐ Yes	Date ____
Hypertension	☒ No	☐ Yes	Jaundice	☒ No	☐ Yes	Date ____
Uremia	☒ No	☐ Yes	HAA Test	☒ Negative	☐ Positive	Date ____

REACTIONS FROM *PREVIOUS* THERAPY?

☒ No ☐ Yes; *please specify therapy and reaction:*

PHYSICAL EXAMINATION

Number and list each positive finding in pre-treatment examination. On post-treatment examination, use same number and indicate any changes in each finding. Use additional numbers to list any new positive findings.

Pre-Treatment	Post-Treatment
None, elective arthroscopy of knee	same

DIAGNOSIS

☐ Prophylaxis ☐ Joint Hemorrhage ☐ Muscle Hemorrhage

☐ Overt Bleeding ☐ Massive Wound ☒ Surgery

CONCOMITANT MEDICATIONS
(In use at enrollment or begun during study)

DRUG	SINGLE DOSE	ROUTE (IM, IV, PO)	SCHEDULE	DATE STARTED	DATE STOPPED
Demerol	50 mg	p.o.	Q4H prn	9/22/77	9/26/77
			AP000780		

ARMOUR000988

ARMO0000092_0202

Date	Hemoglobin	Hematocrit	HAA	Bilirubin	S.G.O.T.	
9/21/77	16.2	45.2	neg	0.9	65	
9/26/77	15.4	43.0	neg	0.8	60	

AL-1259 ADMINISTRATION

Lot No. K852030 Dosage 3942 AHF units in 90 ml

AHF units/vial 1314 Solution Time 20 *(minutes)* Infusion Time 15 *(minutes)*

Calculated AHF Rise 100 % Actual AHF Rise 117 %

	Date	Time	B.P.	Pulse	Temp.	PTT	AHF%
Preinfusion	9/21	8.00 AM	120/70	86	98.6	88	<1
Immediate Post Infusion	9/21	8.10 AM	118/74	86	98.6	41	117
1 hr " "	9/21	9.00 AM	124/76	84	98.6	41	98
4 hr " "	9/21	12.15 PM	120/70	80	98.6	45	41
12 hr " "	9/21	8.20 PM	128/74	86	98.6	52	20
24 hr " "	9/21	8.00 AM	118/78	84	98.6	65	8.4
__ hr " "							

ADVERSE EFFECT

[X] No adverse effects [ ] Adverse effects as in table:

SYMPTOMS OF SIGNS	DATE AND TIME OF ONSET	DURATION	SEVERITY*

*Classify reaction as 1 = mild; 2 = moderate; 3 = severe

Clinical Response No hemorrhage after arthroscopy; surgery uneventful

AP000781

_____, M.D. Date 11/18/77

Signature of Investigator

ANTIHEMOPHILIC FACTOR (HUMAN)
Protocol No. 101

200

Patient Identification [redacted] Study No. 4

Age 38 *(yrs)* Weight 85 *(kg)* or ____ *(lbs)* Height 68 *(in)* Case No. 2

MEDICAL HISTORY

Kidney Disease	[X] No	[ ] Yes	Hepatitis	[X] No	[ ] Yes	Date ____
Hypertension	[X] No	[ ] Yes	Jaundice	[X] No	[ ] Yes	Date ____
Uremia	[X] No	[ ] Yes	HAA Test	[X] Negative	[ ] Positive	Date ____

REACTIONS FROM *PREVIOUS* THERAPY?

[X] No [ ] Yes; *please specify therapy and reaction:*

PHYSICAL EXAMINATION

Number and list each positive finding in pre-treatment examination. On post-treatment examination, use same number and indicate any changes in each finding. Use additional numbers to list any new positive findings.

Pre-Treatment	Post-Treatment
1. Hemophilic Arthropathy, chronic	1. Unchanged
2. Hip Flexion contracture	2. Improved after surgery

DIAGNOSIS

[ ] Prophylaxis [ ] Joint Hemorrhage [ ] Muscle Hemorrhage

[ ] Overt Bleeding [ ] Massive Wound [X] Surgery

CONCOMITANT MEDICATIONS
(In use at enrollment or begun during study)

DRUG	SINGLE DOSE	ROUTE *(IM, IV, PO)*	SCHEDULE	DATE STARTED	DATE STOPPED
Dicloxicillin	500 mg	PO	Q6H	10/24/77	11/10/77

AP000782

ARMOUR000990

ARMO0000092_0204

Date	Hemoglobin	Hematocrit	HAA	Bilirubin	S.G.O.T.	
11/1/77	14.2	41.0	Neg.	0.7	80	
11/15/77	12.0	35.0	Neg.	0.8	88	

AL-1259 ADMINISTRATION

Lot No. K852030 Dosage 2628 AHF units in 60 ml

AHF units/vial 1314 Solution Time 30 *(minutes)* Infusion Time 20 *(minutes)*

Calculated AHF Rise 66 % Actual AHF Rise 72 %

	Date	Time	B.P.	Pulse	Temp.	PTT	AHF%
Preinfusion	11/2	11.30 AM	130/70	76	98.2	85.2	<1
Immediate Post Infusion	11/2	1.05 PM	124/90	72	97.8	44.0	72
1 hr " "	11/2	2.00 PM	120/80	60	98.6	47.8	56
4 hr " "	11/2	5.00 PM	118/76	72	98.2	49.5	36
12 hr " "	11/3	1.00 AM	140/72	88	97.6	56.4	16
24 hr " "	11/3	1.10 PM	120/80	88	98.6	63.9	6
__ hr " "							

ADVERSE EFFECT

[X] No adverse effects [ ] Adverse effects as in table:

SYMPTOMS OF SIGNS	DATE AND TIME OF ONSET	DURATION	SEVERITY*

*Classify reaction as 1 = mild; 2 = moderate; 3 = severe

Clinical Response excellent; uneventful total hip replacement

, M.D. Date 11-18-77

Signature of Investigator

AP000783

ARMOUR000991

ARMO0000092_0205

ANTIHEMOPHILIC FACTOR (HUMAN)
Protocol No. 101

Patient Identification [redacted] Study No. 4

Age 38 *(yrs)* Weight 85 *(kg)* or ____ *(lbs)* Height 68 *(in)* Case No. 3

MEDICAL HISTORY

Kidney Disease	[X] No	[ ] Yes	Hepatitis	[X] No	[ ] Yes	Date ____
Hypertension	[X] No	[ ] Yes	Jaundice	[X] No	[ ] Yes	Date ____
Uremia	[X] No	[ ] Yes	HAA Test	[X] Negative	[ ] Positive	Date ____

REACTIONS FROM *PREVIOUS* THERAPY?

[X] No [ ] Yes; *please specify therapy and reaction:*

PHYSICAL EXAMINATION

Number and list each positive finding in pre-treatment examination. On post-treatment examination, use same number and indicate any changes in each finding. Use additional numbers to list any new positive findings.

Pre-Treatment	Post-Treatment
1. Hemophilic arthropathy, chronic	1. Unchanged
2. Hip flexion contracture	2. Improved after surgery

DIAGNOSIS

[ ] Prophylaxis [ ] Joint Hemorrhage [ ] Muscle Hemorrhage
[ ] Overt Bleeding [ ] Massive Wound [ ] Surgery

CONCOMITANT MEDICATIONS
(In use at enrollment or begun during study)

DRUG	SINGLE DOSE	ROUTE *(IM, IV, PO)*	SCHEDULE	DATE STARTED	DATE STOPPED
Dicloxacillin	500 mg	PO	Q6H	10/24/77	11/10/77

AP000784

ARMOUR000992

ARMO0000092_0206

				Bilirubin	S.G.O.T.	
1/1/77	14.2	41.0	Neg.	0.7	80	
1/15/77	12.0	35.0	Neg.	0.8	88	

AL-1259 ADMINISTRATION

Lot No. K 852030 Dosage 1314 AHF units in 30 ml

AHF units/vial 1314 Solution Time 25 *(minutes)* Infusion Time 20 *(minutes)*

Calculated AHF Rise 32 % Actual AHF Rise 42 %

	Date	Time	B.P.	Pulse	Temp.	PTT	AHF%
Preinfusion	11/6	8 AM	130/74	84	99.0	47	32
Immediate Post Infusion	11/6	8.10 AM	132/70	88	99.0	43	74
1 hr " "							
4 hr " "							
12 hr " "							
24 hr " "							
__ hr " "							

ADVERSE EFFECT

☐ No adverse effects ☐ Adverse effects as in table:

SYMPTOMS OF SIGNS	DATE AND TIME OF ONSET	DURATION	SEVERITY*

*Classify reaction as 1 = mild; 2 = moderate; 3 = severe

Clinical Response excellent; no bleeding after total hip replacement

AP000785

_____, M.D. Date 11/18/77

Signature of Investigator

AL-1259
ANTIHEMOPHILIC FACTOR (HUMAN)
Protocol No. 101

Patient Identification [redacted] Study No. 4

Age 13 (yrs) Weight 30 (kg) or ____ (lbs) Height 51 (in) Case No. 4

MEDICAL HISTORY

Kidney Disease	[X] No	[ ] Yes	Hepatitis	[X] No	[ ] Yes	Date ____
Hypertension	[X] No	[ ] Yes	Jaundice	[X] No	[ ] Yes	Date ____
Uremia	[X] No	[ ] Yes	HAA Test	[X] Negative	[ ] Positive	Date ____

REACTIONS FROM *PREVIOUS* THERAPY?

[X] No [ ] Yes; *please specify therapy and reaction:*

PHYSICAL EXAMINATION

Number and list each positive finding in pre-treatment examination. On post-treatment examination, use same number and indicate any changes in each finding. Use additional numbers to list any new positive findings.

Pre-Treatment	Post-Treatment
Swelling (L) Knee	Swelling decreased

DIAGNOSIS

[ ] Prophylaxis [X] Joint Hemorrhage [ ] Muscle Hemorrhage
[ ] Overt Bleeding [ ] Massive Wound [ ] Surgery

CONCOMITANT MEDICATIONS
(In use at enrollment or begun during study)

DRUG	SINGLE DOSE	ROUTE (IM, IV, PO)	SCHEDULE	DATE STARTED	DATE STOPPED
Tylenol	300 mg	PO	Q4H prn	1/6/78	1/10/78

AP000786

Date	Hemoglobin	Hematocrit	HAA	Bilirubin	S.G.O.T.
1/6/78					

AL-1259 ADMINISTRATION

Lot No. K852030 Dosage 1314 AHF units in 30 ml

AHF units/vial 1314 Solution Time 20 *(minutes)* Infusion Time 10 *(minutes)*

Calculated AHF Rise 88 % Actual AHF Rise 86 %

	Date	Time	B.P.	Pulse	Temp.	PTT	AHF%
Preinfusion	1/7/78	8.30 AM	100/65	76	98.6	74	4%
Immediate Post Infusion	1/7	8.40 AM	100/60	76	98.4	40	90%
1 hr " "							
4 hr " "							
12 hr " "							
24 hr " "							
__ hr " "							

ADVERSE EFFECT

[X] No adverse effects [ ] Adverse effects as in table:

SYMPTOMS OF SIGNS	DATE AND TIME OF ONSET	DURATION	SEVERITY*

*Classify reaction as 1 = mild; 2 = moderate; 3 = severe

Clinical Response BLEEDING ARRESTED

AP000787

_____, M.D. Date 1/27/78

Signature of Investigator

Protocol No. 101 206

Patient Identification [redacted] Study No. 4

Age 24 (yrs) Weight 54 (kg) or ____ (lbs) Height 69 (in) Case No. 5

MEDICAL HISTORY

Kidney Disease	[X] No	[ ] Yes	Hepatitis	[X] No	[ ] Yes	Date ____
Hypertension	[X] No	[ ] Yes	Jaundice	[X] No	[ ] Yes	Date ____
Uremia	[X] No	[ ] Yes	HAA Test	[X] Negative	[ ] Positive	Date ____

REACTIONS FROM *PREVIOUS* THERAPY?

[X] No [ ] Yes; *please specify therapy and reaction:*

PHYSICAL EXAMINATION

Number and list each positive finding in pre-treatment examination. On post-treatment examination, use same number and indicate any changes in each finding. Use additional numbers to list any new positive findings.

Pre-Treatment	Post-Treatment
Swelling (R) Knee	swelling improved

DIAGNOSIS

[ ] Prophylaxis [X] Joint Hemorrhage [ ] Muscle Hemorrhage

[ ] Overt Bleeding [ ] Massive Wound [ ] Surgery

CONCOMITANT MEDICATIONS
(In use at enrollment or begun during study)

DRUG	SINGLE DOSE	ROUTE (IM, IV, PO)	SCHEDULE	DATE STARTED	DATE STOPPED
			AP000788		

ARMOUR000996

ARMO0000092_0210

Date	Hemoglobin	Hematocrit	HAA	Bilirubin	S.G.O.T.	
1/4/77	13.8	39	Neg.	0.7	45	

AL-1259 ADMINISTRATION

Lot No. K852030 Dosage 1314 AHF units in 30 ml

AHF units/vial 1314 Solution Time 18 *(minutes)* Infusion Time 5 *(minutes)*

Calculated AHF Rise 48 % Actual AHF Rise 55 %

	Date	Time	B.P.	Pulse	Temp.	PTT	AHF%
Preinfusion	1/6/77	8:30 AM	130/84	84	98.8	68	6
Immediate Post Infusion	1/6/77	8:40 AM	134/84	84	98.8	44	61
1 hr " "							
4 hr " "							
12 hr " "							
24 hr " "							
__ hr " "							

ADVERSE EFFECT

[X] No adverse effects [ ] Adverse effects as in table:

SYMPTOMS OF SIGNS	DATE AND TIME OF ONSET	DURATION	SEVERITY*

*Classify reaction as 1 = mild; 2 = moderate; 3 = severe

Clinical Response Excellent; bleeding controlled

AP000789

_____, M.D. Date 1/27/78

Signature of Investigator

Protocol No. 101

208

Patient Identification [redacted] Study No. 4

Age 33 (yrs) Weight 71 (kg) or ____ (lbs) Height 70 (in) Case No. 6

MEDICAL HISTORY

Kidney Disease	[X] No	[ ] Yes	Hepatitis	[X] No	[ ] Yes	Date ____
Hypertension	[ ] No	[X] Yes	Jaundice	[X] No	[ ] Yes	Date ____
Uremia	[X] No	[ ] Yes	HAA Test	[X] Negative	[ ] Positive	Date ____

REACTIONS FROM *PREVIOUS* THERAPY?

[X] No [ ] Yes; *please specify therapy and reaction:*

PHYSICAL EXAMINATION

Number and list each positive finding in pre-treatment examination. On post-treatment examination, use same number and indicate any changes in each finding. Use additional numbers to list any new positive findings.

Pre-Treatment	Post-Treatment
Fracture (R) femur	same; no hemorrhage

DIAGNOSIS

[X] Prophylaxis [ ] Joint Hemorrhage [ ] Muscle Hemorrhage

[ ] Overt Bleeding [ ] Massive Wound [ ] Surgery

CONCOMITANT MEDICATIONS
(In use at enrollment or begun during study)

DRUG	SINGLE DOSE	ROUTE (IM, IV, PO)	SCHEDULE	DATE STARTED	DATE STOPPED
Hygroton	50 mg	PO	once daily	11/77	

AP000790

ARMOUR000998

Date	Hemoglobin	Hematocrit	HAA	Bilirubin	S.G.O.T.	
1/26/78	14.1	41	Neg.	0.8	60	

AL-1259 ADMINISTRATION

Lot No. K852030 Dosage 1314 AHF units in 30 ml

AHF units/vial 1314 Solution Time 20 *(minutes)* Infusion Time 8 *(minutes)*

Calculated AHF Rise 38 % Actual AHF Rise 49 %

	Date	Time	B.P.	Pulse	Temp.	PTT	AHF%
Preinfusion	1/27/78	8:00 AM	140/80	80	98.8	55	9
Immediate Post Infusion	1/27/78	8:10 AM	144/80	84	98.6	45	58
1 hr " "	1/27	9:05 AM	136/82	80	98.6	44	52
4 hr " "	1/27	noon	140/80	80	98.6	45	33
12 hr " "	1/27	2:00 PM	144/84	86	98.8	47	26
24 hr " "	1/28	8:00 AM	140/84	76	98.8	54	8.5
__ hr " "							

ADVERSE EFFECT

[X] No adverse effects [ ] Adverse effects as in table:

SYMPTOMS OF SIGNS	DATE AND TIME OF ONSET	DURATION	SEVERITY*

*Classify reaction as 1 = mild; 2 = moderate; 3 = severe

Clinical Response No hemorrhage

AP000791

_____, M.D. Date 1/31/78

Signature of Investigator

ARMOUR000999